Enhancement of the efficacy of the parasitoid, *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) by provision of floral resources to improve biological control of its host, the Argentine stem weevil (*Listronotus bonariensis*) (Kuschel) (Coleoptera: Curculionidae)

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By
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In this study, conservation biological control principles were applied to increase the efficacy of *Microctonus hyperodae* Loan (Hymenoptera: Braconidae), a parasitoid of the Argentine stem weevil (*Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae)). The Argentine stem weevil is a serious pest in New Zealand pasture.

Seven flowering plant species were selected for laboratory experiments to assess the best plant species to increase the longevity of *M. hyperodae*. Longevity of the parasitoid was significantly increased with buckwheat (*Fagopyrum esculentum* Moench) compared with six other flowering plants. Floral architecture measurements and nectar quality analysis were used to understand the floral selectivity and consequent 'preferences' of the parasitoids. A wider corolla aperture and a shorter corolla depth, as well as the sucrose/(glucose+fructose) ratio of the nectar were positively correlated with the longevity of *M. hyperodae*. These results were compared with those for other parasitoids.

Lifetime carbohydrate reserves were analysed in continuously-fed and unfed (water control) *M. hyperodae* in the laboratory. Total sugar levels (as a measure of nutritional state) decreased continuously throughout unfed parasitoids' lifespans, whereas in continuously-fed individuals, levels increased initially and then decreased. Glucose dominated in the unfed parasitoids while glucose and fructose dominated in the fed individuals. The common insect haemolymph sugar, trehalose, was not detected in unfed *M. hyperodae*, but was detected in fed parasitoids. Parasitoids that fed from buckwheat in the laboratory showed higher sucrose levels immediately after feeding, but these declined to the levels observed in honey-fed individuals after one hour.

The fructose/total sugars (f/t) ratio was clearly different between the fed and unfed *M. hyperodae*. This ratio was a good indicator for distinguishing fed from unfed parasitoids and was therefore a useful tool for understanding the feeding history of *M. hyperodae*.

Buckwheat was deployed in the field to assess parasitoid abundance at different distances from the floral resources. The highest number of *M. hyperodae* occurred near buckwheat plots. The number rapidly declined with increasing distance and was at its lowest at 7 to 8m, followed by increasing numbers. Parasitoid numbers significantly increased near buckwheat plots compared with the middle and furthest distances.

Field-collected parasitoids were analysed to determine their nutritional state and feeding history. A high proportion (79%) of parasitoids collected near buckwheat plots had fed and, therefore, had an increased total sugar level and f/t ratio. Only 20% collected from grasses on road-side verges apparently had access to sugar sources. The f/t ratio in combination with total sugar levels was a useful tool to understand the nutritional state and feeding history of field-collected parasitoids.

The effect of buckwheat deployment in the field on parasitism rates of *L. bonariensis* was measured. Parasitism rates of the first summer generation of *L. bonariensis* near buckwheat plots were increased by 250% compared with the controls. Parasitism rates of the second summer generation of *L. bonariensis* did not differ between those near buckwheat and the controls.

Key words: conservation biological control, parasitoid, *Microctonus hyperodae, Listronotus bonariensis,* buckwheat, floral architecture, nectar quality, sucrose/(glucose+fructose) ratio, fructose/total sugar (f/t) ratio, total sugar level, nutritional state, feeding history, parasitism rate.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

CHAPTER 1

GENERAL INTRODUCTION

1.1 Biological control

Biological control has been used to manage pests for more than 100 years (Hawkins *et al.* 1999). It uses natural enemies to reduce pest populations, thereby reducing damage to a manageable level (Samways 1981). This has been one of the best options for modern pest management in the light of negative environmental consequences of pesticides (van Emden & Peakall 1996; Williams 1997; Paoletti & Pimentel 2000). However, since the first successful introduction of the vedalia beetle (*Rodolia cardinalis* Mulsant) in 1888 to control cottony cushion scale (*Icerya purchasi* Maskell) in California, only a 10% success rate has been recorded in classical biological control of arthropods by arthropods (see 1.1.1 below), with no major improvement in success rates over the years (Greathead & Greathead 1992; Gurr & Wratten 2000; Gurr *et al.* 2000).

1.1.1 Methods of biological control

There are three main methods of biological control: classical, inundative and conservation. Classical biological control involves finding a suitable natural enemy, usually within the target pest's natural geographic distribution, rearing and screening it in the laboratory, then releasing it into new regions. The intention is for the released agent to establish self-sustaining populations that will suppress the target pest in perpetuity. Inundative biological control involves the release of a large number of natural enemies to gain immediate results (van Driesche & Bellows 1996), but such releases may need to be made repeatedly. Conservation biological control focuses on habitat manipulation and pesticide reduction to enhance the efficacy of existing natural enemies (Ehler 1988; Gurr *et al.* 2000; Landis *et al.* 2000; Gurr *et al.* 2004), which is the main focus of this thesis.

1.1.2 Conservation biological control

Ehler (1988) stated that conservation biological control (CBC) involves 'actions that preserve or protect natural enemies'. In CBC, food webs are engineered to increase the efficacy of existing natural enemies, potentially reducing noxious conditions (such as chemical use) or enhancing favourable ones. This enhancement can be achieved by providing an appropriate diversity of alternative food sources, shelter, microclimates and alternative prey, or hosts (White *et al.* 1995; Hickman & Wratten 1996; Landis *et al.* 2000). Appropriate diversity means that floral resources (pollen and nectar) are provided to benefit the natural enemies without benefiting the pest (Speight 1983; van Emden 1990; Gurr *et al.* 1998; Baggen *et al.* 1999), and without using plant species which cause agronomic problems in agricultural fields. If this balance can be demonstrated in field situations, then it can be said that 'ecological engineering' (Odum 1962) has been successfully practised.

There were some observations in the past that reported the presence of flowering plants increased the parasitism rate and biological control (Wolcott 1942; Allen & Smith 1958). Subsequent field experiments also showed that the parasitism rate of the targeted pest has been increased by provision of floral resources in the vicinity of orchards (Chumakova 1960; Powell 1986). Further successful research in the laboratory and in field situations (Leius 1960, 1961, 1967) extended the knowledge and the possibility of implementing CBC programmes. The 'parasitoid nectar provision hypothesis' (Heimpel & Jervis 2005) elaborates an in-depth analysis of the mechanisms involved in this process. The main assumption in this hyphothesis is that sugar is a limiting factor in agricultural and other man made systems and, therefore, parasitoids do not have the opportunity to obtain sufficient sugar meals from these systems. Fundamentally, the approach of the nectar provision hypothesis is similar to that of Wratten *et al.* (2003), but it focuses on steps two to five of conservation biological control hierarchical levels (see section 1.7, p. 14; Wratten *et al.*2003).

Provision of appropriate shelter for natural enemies is also very important, so that they can remain from year to year in crop fields to help manage pest populations. Among the successful attempts to provide shelter for natural enemies is the use of 'beetle banks' that consist of perennial grasses sown on a raised earth bank (Thomas *et al.* 1991, 1992; Wratten 1992; Landis

et al. 2000; MacLeod *et al.* 2004). Recently, an increased effort has been made to study the ecology of pests and their natural enemies, to conserve or enhance the efficacy of natural enemies (Ehler 1988; Gurr *et al.* 2000; Landis *et al.* 2000; Gurr *et al.* 2003; Gurr *et al.* 2004).

1.1.3 Provision of floral resources

The importance of floral resources to the effectiveness of natural enemies in suppression of pests is well documented (Takasu & Lewis 1996; Baggen & Gurr 1998). Numerous parasitoid species have been reported feeding on a broad range of flowers (Jervis *et al.* 1993). Floral nectar contains sugars, proteins, amino acids, lipids and many other organic and inorganic substances (Hagen 1986). For many insects, it is vital to have access to floral resources to mature their eggs (Jervis & Kidd 1996), and the provision of floral resources can increase their longevity and fecundity (Baggen & Gurr 1998; Irvin 1999; Berndt *et al.* 2002). Laboratory experiments have shown that sugar can increase the longevity and fecundity of parasitoids (Hodgson *et al.* 1993; Wäckers 2001; Gurr *et al.* 2004; Berndt & Wratten in press), and can even change their population sex-ratios (Berndt & Wratten 2005). Also, parasitoid host searching activity (Takasu & Lewis 1996; Landis *et al.* 2000) and parasitism rates can be increased in the field (Leius 1967) by provision of sugar sources. Although floral resources have been used with many vegetable and horticultural crops to help manage insect pests, no information is available on using them to manage pests in pasture. However, pasture is the largest 'monoculture' in New Zealand and major benefits could be gained if research showed the provision of floral resources was effective in this situation.

1.2 The pasture industry in New Zealand

Fifty-three percent of New Zealand export earnings are from agricultural products, excluding forestry (InFos Data Base, 2002). Pasture comprises 38% of New Zealand land cover (Land Cover Data Base, 1997) and accounts for the largest single land use in this country. Dairying, sheep farming and beef cattle are the main industries dependent on pastures while, in recent years, deer farming has also become important.

New Zealand has 10 times more sheep than people (New Zealand Official Yearbook 2004). Over time, grassland quality in New Zealand has greatly improved, and throughout the year the best sheep farms can accommodate about 25 sheep/ha and dairy farms can carry 2.5 cows/ha (New Zealand Official Yearbook 2004). Agricultural exports accounted for NZ$18,823 million in 2003 and, of these, dairy, meat and wool and other pasture-related products comprised 57% (NZ$10,686 million) (New Zealand Official Yearbook 2004).

1.2.1 Pastoral plant communities

Although more than a hundred plant species are found in natural grasslands in New Zealand (Kemp *et al.* 1999), not many of them are suitable for managed pastures. There are about 30 plant species used in managed pastures. Only a few of them are widely used. (White & Hodgson 1999). The major pasture species found in managed grasslands are perennials and, perennial ryegrass (*Lolium perenne* L. (Cyperales: Poaceae)) and white clover (*Trifolium repens* L. (Fabales: Fabaceae)) are most commonly grown. However, some regions that experience prolonged dry and cold conditions require annual species instead. Species that are not in common use may have special applications in different environmental conditions that could facilitate the survival and productivity of those species in difficult areas.

Farmers may want to change pasture species to manipulate the annual distribution of herbage output, optimise nutritional value, improve persistence, increase the resistance to pests and diseases, improve compatibility with weeds, enhance drainage and to maintain a flat field surface. All of these characteristics are considered to be important in selecting plant species for pastures. Therefore, the decision to select a mixture of plants is based on the relative contributions of candidate pasture plants to the above outcomes. Although there may be up to 30 plant species present in pastoral land, more than 80% of the herbage is produced by fewer than three species (White & Hodgson 1999).

Perennial ryegrass and Italian ryegrass (*Lolium multiflorum* Lam. (Cyperales: Poaceae)) are the main grass species sown in New Zealand pastures. There are many cultivars (hybrids between these two species) of ryegrass on the market and 10 of them are commonly used. The relative attributes of these cultivars change from annual (short) to perennial (long) rotation. For example,

persistence of the grass increases as cultivars change from annual to perennial types, but the reverse is true for nutritive value and growth during the winter months (Kemp *et al.* 1999).

1.3 Pasture pests and the Argentine stem weevil

Many pathogens and pests occur in New Zealand pastures, including the Argentine stem weevil (*Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae)), the clover root weevil (*Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae)), grass grub (*Costelytra zealandica* White (Coleoptera: Scarabaeidae)), porina (*Wiseana spp* (Lepidoptera: Hepialidae)), the black beetle (*Heteronychus arator* F. (Coleoptera: Scarabaeidae)), the black field cricket (*Teleogryllus commodus* Walker (Orthoptera: Gryllidae)) and army worm (*Mythimna separata* Walker (Lepidoptera: Noctuidae)). *L. bonariensis*, grass grub and porina are persistent and found in many pastures in the country, while the other species are sporadic and show localized distributions (White & Hodgson 1999). Cutworm (*Agrotis ipsilon aneituma* Walker (Lepidoptera: Noctuidae)) and a number of other moth species with minor pest status affect ryegrass (Prestidge *et al.* 1994). However, *L. bonariensis* is considered to be the most damaging pest of pasture in New Zealand (Pottinger 1961a; Prestidge *et al.* 1991). Estimated damage to the pasture industry by *L. bonariensis* is somewhere between NZ$78- 251 million per year (Prestidge *et al.* 1991).

L. bonariensis originates from southern South America, and has been recorded from Argentina, Uruguay, Chile, and Bolivia (Barker & Pottinger 1982). A molecular comparison of *L. bonariensis* from New Zealand, Australia and South America showed that it originated from Rio de la Plata, on the coast of Argentina (Lenney-Williams *et al.* 1994). The first collection of *L. bonariensis* in New Zealand was made by Miller in 1927 (Marshall 1935). Morrison (1935) recorded *L. bonariensis* from wheat, barley and ryegrass in New Zealand, while subsequent records also showed that it was present in pasture (Morrison 1935, 1938, 1939; Morrison & Blair 1949). Although the literature shows that *L. bonariensis* has been present in New Zealand for a long period, it was not recognised as a pest until the late 1950s (Kelsey 1958; Pottinger 1961b).

L. bonariensis has been recorded damaging wheat in Brazil and Argentina, and also sports turf in Australia (Goldson *et al.* 1998a). *L. bonariensis* is considered to be a pest of Gramineae (Poaceae) in New Zealand, and is mainly a pest of pasture (Barker *et al.* 1984a). Goldson *et al.*

(1998b) suggested that *L. bonarensis* has become a highly destructive pasture pest in New Zealand due to the absence of its natural enemies in this country.

Kelsey (1958) showed that *L. bonariensis* damages grass in three different ways: adults feed on leaves, larvae tunnel into tillers and adults injure seed heads. The damage caused by larvae (Plate 1.1a) is much greater than that caused by adults (Plate 1.1b). Although *L. bonarensis* adults feed on leaves, causing total loss of leaves in scattered patches in grasslands, this damage could be recovered if no further damage occurred due to tiller mining by larvae (Kelsey 1958). This study also showed that the highest level of damage to grassland due to seed-head injury was 7%, which is low compared with tiller mining, which makes grass wilt, discolour and eventually die. In this thesis the success of CBC applications in pastures were investigated by provision of floral resources to increases the efficacy of the parasitoid of *L. bonariensis*.

1.4 Population biology of *L. bonariensis* and its control measures

1.4.1 Biology of *L. bonariensis*

The female weevil deposits 1 - 8 eggs in the leaf cuticle in the ryegrass (Pottinger 1961a; Goldson *et al.* 1998a) and the newly hatched larvae tunnel into the grass tillers (Barker *et al.* 1984a). More than one larva can feed on a tiller, particularly during the early instars (Barker *et al.* 1984b). The stem-mining larvae are reported to feed on 3-8 ryegrass tillers before pupating into the soil (Goldson *et al.* 1998a).

L. bonariensis is active throughout the summer in New Zealand (Goldson *et al.* 1998b). It has been reported that it completes two generations each summer in the South Island (Barker & Pottinger 1982), and three may occur in the warmer parts of the North Island (Pottinger 1961b). The eggs and larvae of the first summer generation were more abundant than those of the second (Barker & Pottinger 1982).

Adults of *L. bonariensis* overwinter in a state of photoperiodically-induced diapause. Egg-laying commences in late September, in the spring (Goldson *et al.* 1998b). The remaining overwintered population tends to die in early December. The lowest weevil population was recorded on 9

December (Phillips *et al.* 1998). A five year study conducted in the Lincoln AgResearch farm indicated that the same levels of low weevil population were observed around early December throughout that study (Phillips unpublished).

1.4.2 Control measures

Pesticides have not been effective in controlling *L. bonariensis* for several reasons. *L. bonariensis*'s rapid reproduction, protected stem-mining larvae and the flight capacity of adults (especially in dry areas) (Pottinger 1966; Goldson & Emberson 1981) reduces the potential to control this pest using chemicals (Goldson *et al.* 1994). However, some farmers use chemically treated grass seeds to protect new cotyledons from being attacked by *L. bonariensis* (Goldson *et al.* 1994). Several granular systemic products also have been used by farmers at drilling to minimise pest damage.

Endophytes and resistant grasses

Due to the low efficacy of pesticides for controlling *L. bonariensis*, alternative methods of control have been sought. Development of grasses resistant to *L. bonariensis* has been a major focus (White & Hodgson 1999). Comparisons of the susceptibility of short rotation ryegrass (*L. multiflorum*) with perennial ryegrass (*L. perenne*) showed considerable differences. Eighty-four percent of *L. multiflorum* tillers were attacked by *L. bonariensis* larvae compared with only 35% of *L. perenne* (Timlin 1964). There is an inverse relationship between the number of *L. bonariensis* oviposition sites and the diameter of the grass stem (Goldson *et al.* 1982; Pilkington & Springett 1988). Subsequent experiments demonstrated a correlation between the level of weevil attack and the fibre, silica and soluble carbohydrate content of tillers (Barker 1989).

The impact of the endophyte fungus *Neotyphodium lolii* (Glenn, Bacon & Hanlin) (Hypocreales: Clavicipitaceae) on *L. bonariensis*'s activity on ryegrass was observed in the 1980s (Fletcher & Harvey 1981). Barker (1989) found that the *L. bonariensis*'s ability to feed on grass was dependent on the availability of non-endophyte infected grass. The discovery of a negative effect of *N. lolii* on *L. bonariensis*'s impact on pasture led farmers to use *N. lolii* infected ryegrass seeds for sowing. However, concerns arose as a result of grass staggers and other negative metabolic effects on livestock. The presence of the endophyte led to very complex effects that were not

clearly understood in the early stages of the research (Fletcher *et al.* 1990). *N. lolli* produces three major toxins: peramine, which deters feeding, and oviposition by *L. bonariensis* and some other pests. Lolitrem B which causes "ryegrass staggers" in grazing animals, and, ergovaline causes heat stress and blood circulation problems in grazing animals (Charlton & Stewart 2000). Damage caused to livestock can be minimised by sowing endophyte-free ryegrass cultivars, other grass species, or low-endophyte cultivars. High-endophyte cultivars contain over 70% of endophyte and low endophyte cultivars contain about 5% (Charlton & Stewart 2000). Recently, new endophyte species have been introduced to overcome the staggers problem. A new endophyte, AR1, was introduced recently and that produces peramine which provides resistance to the weevil, but lacks the mammalian toxins ergovaline and lolitrem B (Popay & Baltus 2001). The long-term value of this for *L. bonariensis* control is unknown.

Biological control of **L. bonariensis**

L. bonariensis has established in New Zealand without its associated natural enemies. Grass resistance and the endophyte-infected grasses partially fulfilled the low cost and environmentally friendly management requirements for *L. bonariensis*. The first attempt at biological control of *L. bonariensis* was made in the 1960s. The Department of Scientific and Industrial Research introduced an egg parasitoid *Patasson* (=*Anaphes*) *atomarius* Brethes (Hymenoptera: Mymaridae) and an unidentified parasitoid larva as biological control agents, but both agents failed to establish in New Zealand (Dymock 1989). Subsequently, *Microctonus hyperodae* Loan (Hymenoptera: Braconidae), a candidate biological control agent first described by Loan & Lloyd (1974) as potentially suitable for New Zealand, was selected for laboratory testing before being released in the field (Goldson *et al.* 1992).

1.5 *M. hyperodae* as a biological control agent

The koinobiont, solitary endoparasitoid, *M. hyperodae* was identified as a candidate biological control agent for *L. bonariensis*, its only known host (Loan & Lloyd 1974). *M. hyperodae* attacks the adult stage of *L. bonariensis* (Plate 1.1c). After oviposition, adult hosts remain active while the parasitoid egg hatches and the larva begins to develop. The fully developed larva then emerges from the host to pupate (Plate 1.1d), thereby killing the weevil (Loan & Lloyd 1974).

Only females emerged from parasitised weevils, indicating that the species is thelytokous (Loan & Lloyd 1974).

1.5.1 Biology of *M. hyperodae*

M. hyperodae was first found in 1961 as a parasitoid of adult *Hyperodes* (=*Listronotus*) *bonariensis* at Colonia, Uruguay, which is a native species of southern South America (Loan & Lloyd 1974). Loan & Lloyd (1974) were the first to describe the taxonomy and biology of this parasitoid and its development within its host.

M. hyperodae females reach the maximum egg laying potential within 24 hours after emergence (Phillips *et al.* 1996) and are capable of ovipositing between 30 to 60 eggs in several adult weevils (McNeill *et al.* 1993; Barlow *et al.* 1994). The larva develops within the living host until maturity (McNeill *et al.* 1993; Phillips *et al.* 1996). The mature larva then emerges to pupate, while the host dies (Phillips *et al.* 1996).

During winter, the parasitoid enters a photoperiodically-induced diapause irrespective of the host's condition (Goldson 1998a) as an arrested egg or larva (McNeill *et al.* 1993; Barlow *et al.* 1994). Development resumes during spring with the increase in temperature. *M. hyperodae* probably completes three generations per year (Goldson *et al.* 1998a). Adult parasitoids are found in the field in late November through to May (McNeill *et al.* 1993; Barlow *et al.* 1994). It seems likely that each adult generation lives less than two weeks in the field, since their longevity under laboratory conditions was 17 days (Phillips 1998).

1.5.2 Egg load of the parasitoid

M. hyperodae is described as a pro-ovigenic species (Goldson *et al.* 1995; Phillips *et al.* 1998). This means that a female has a full egg complement fixed at emergence and it does not develop any more eggs during their lifespan. This is being supported by equal levels between egg load and fecundity in most parasitoids studied. Moreover, egg load did not increase with increasing

age of the parasitoid. (Phillips & Baird 2001). In contrast, synovigenic parasitoid species are able to mature eggs throughout their reproductive life (Jervis *et al.* 2001).

It is estimated that field collected *M. hyperodae*'s pre-oviposition mean egg load is 67 eggs (Phillips *et al.* 1998). In contrast, parasitoids reared in the laboratory had a mean egg load of 47 (Phillips & Baird 2001), which corresponded well with the 48 eggs that were estimated by Goldson *et al.* (1995). Furthermore, newly-emerged parasitoids from the weevils that were collected from the field, and subsequently reared in the laboratory, had a mean egg load of 47 eggs. (Phillips & Baird 2001). This variation between field-collected and laboratory-reared parasitoids suggests that unidentified environmental factors have a major influence on parasitoid egg load.

1.5.3 Introduction of *M. hyperodae* to New Zealand pasture

Thirteen thousand adult weevils were imported, and 247 parasitoids were reared from them in quarantine during 1989 – 1990 (Goldson *et al.* 1990; Phillips & Baird 1996). Permission for field release was granted for the parasitoid after an environmental impact assessment was made, following quarantine and host specificity testing in the laboratory (Goldson *et al.* 1992). *M. hyperodae* was originally reared from weevils collected from eight locations in four South American countries: Argentina (Ascasubi, Mendoza, General Roca and S. C. de Bariloche), Brazil (Porto Alegre), Chile (Concepcion and La Serena) and Uruguay (Colonia) (Figure 1.1 a). Approximately equal number of parasitoids from each location (Goldson *et al.* 1993) were released across New Zealand Figure 1.1 b). All release records were kept for future reference (Phillips & Baird 1996, 2001).

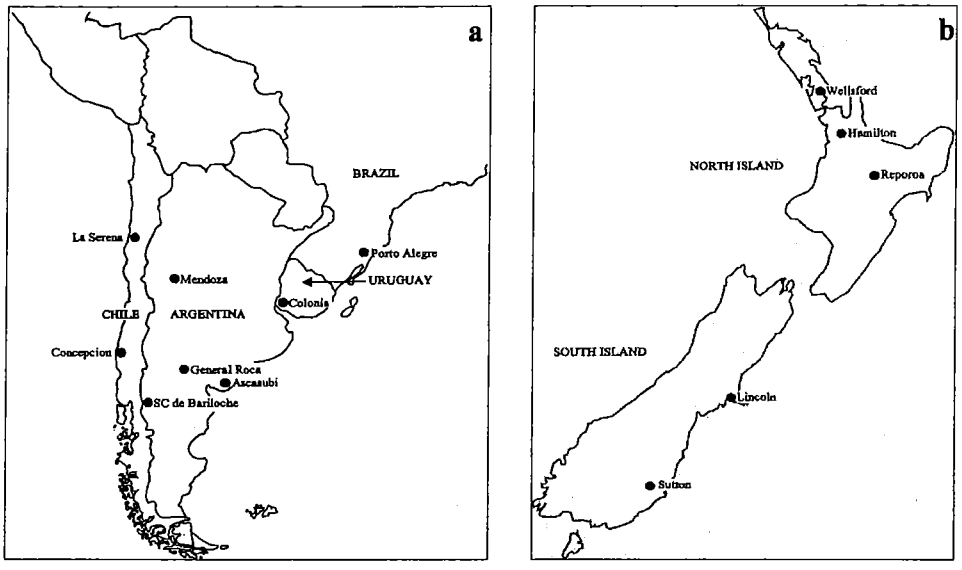

Figure 1.1: a) Map showing the eight original collection sites of *M. hyperodae*. The Andes form the border between Chile and Argentina. b) Map showing the original release sites of *M. hyperodae* in New Zealand in 1991 (reproduced from Phillips *et al.* 1997).

1.5.4 Genetic variation in *M. hyperodae*

A morphometric analysis of *M. hyperodae* showed that parasitoids introduced from east of the Andes (Brazil, Uruguay and Argentina) have out-competed the ones originating from Chile (Phillips *et al.* 1997; Iline & Phillips 2004; Winder *et al.* 2005). A possible reason was due to variation in fecundity, since *M. hyperodae* from Colonia had parasitised significantly more weevils than parasitoids from Porto Alegre, La Serena and S. C. de Bariloche (Goldson *et al.* 1995). Phillips & Baird (2001) suggested that this variation could have been due to the following differences in fecundity: egg load, searching efficiency, egg survival and longevity. They also stated that that geographic variation in egg load was the most likely reason because: 1) Searching efficiency can not be a limiting factor when high numbers of hosts were made available (by Goldson *et al.* 1995), 2) Seventy five generations reared in the laboratory did not show evidence of geographic variation in egg load, 3) There was no geographic variation in longevity (Goldson *et al.* 1995). Therefore, the geographic variation in fecundity reported by Goldson *et al.* (1995) was due to the geographic variation in egg load has been accepted (Phillips & Baird 2001).

1.5.5 Successes and challenges for *M. hyperodae* in the field situations.

The first recovery of *M. hyperodae* from release sites was made in 1992, and considerable parasitism of *L. bonariensis* had occurred by February 1993 at Lincoln. Rates of parasitism of *L. bonariensis* during the winters of 1992 - 1996 were 8.8, 38.5, 53.8, 70 and 72.4%, respectively (Goldson *et al.* 1998b). However, only 6 – 8% of overwintered parasitoid larvae became adults suggesting that 92 – 94% of the parasitoids die during this period (Phillips *et al.* 1998). The reason for this high mortality is yet to be determined. Phillips *et al.* (1998) suggested that adult weevils, which are scarce in spring, could die before the completion of larval parasitoid development and indicated that this type of mortality could be difficult to manipulate. Other possible sources of mortality suggested by Phillips *et al.* (1998) included hyperparasitism (one larvae can survive), pre-pupal and pupal mortality due to low temperature, low humidity, trampling by stock, disease, flooding or predation by other arthropods.

1.6 Enhancement of the efficacy of the biological control agent

A preliminary study to explore the potential to enhance the efficacy of *M. hyperodae* showed that overwintered parasitoids laid only 15% of their egg load from mid-November to mid-December due to a scarcity of hosts during this period (Phillips *et al.* 1998). *L. bonariensis*'s first summer generation begins to emerge from the second week of December in the field and become abundant in January. *L. bonariensis* and *M. hyperodae* showed the same population dynamics in a five year study conducted between 1996 and 2000 at the same site at the Lincoln AgResearch farm (Phillips unpublished). It seems that many adult parasitoids die before the hosts become abundant (Figure 1.2). Therefore, if parasitoid longevity could be extended until the hosts become abundant, then there is a strong possibility that parasitism rates may increase in January (Phillips *et al.* 1998) (Figure 1.2) and thereby increase the effectiveness of biological control.

Phillips *et al.* (1998) estimated that if the number of parasitoids surviving until mid December in the field increased by 20%, then parasitism should increase by 31%. Further, they stated that it could be possible to increase *M. hyperodae* longevity in the field by provision of appropriate liquid food. Currently, there is no information available on the use of food sources by adult *M.*

hyperodae in the field. In laboratory conditions, the longevity of *M. hyperodae* can be increased significantly by provision of liquid sugars (Hodgson *et al.* 1993; Phillips 1998).

M. hyperodae emerges with its final egg load with oogensis occurring during adult eclosion and there is no subsequent increase in egg load over the lifespan of fed parasitoids (Phillips & Baird 2001). This indicates that the provision of floral resources is unlikely to increase *M. hyperodae*'s egg complement. However, as adult parasitoids do not achieve their full potential fecundity in early summer it may be that provision of nectar can increase longevity, and therefore realised fecundity.

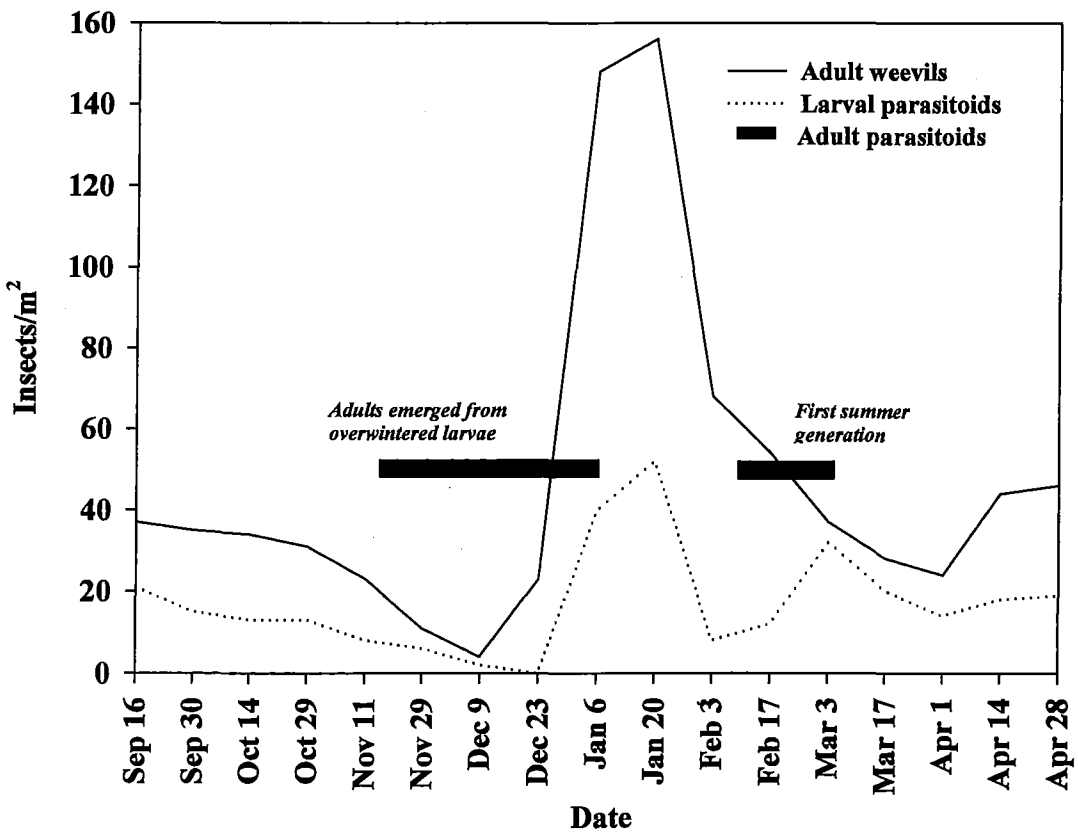

Figure 1.2: The black horizontal bars represent adult parasitoids emerged from overwintered larvae and the first summer generations of adult *M. hyperodae* in the field. First generation adults die before laying many of their eggs because the weevil population is very low during this period. If some of these parasitoids can live until weevils are abundant, there is a possibility that those parasitoids could lay more eggs and parasitize more weevils. In contrast, parasitoids of the first summer generation do not experience this problem (Modified from Phillips *et al.* 1998).

This proposed research aims to enhance the longevity of *M. hyperodae* and thereby increase the realised fecundity in the field. This will be done by the provision of appropriate floral resources in the field to provide the carbohydrate requirements of the adult parasitoid.

1.7 The application of CBC to increase the efficacy of *M. hyperodae*

The selection of appropriate floral resources is the first step towards a successful CBC programme. This involves screening flowering plant species in the laboratory to measure the longevity and fecundity of a biocontrol agent. Flower architecture (Patt *et al.* 1999; Wäckers 2004), nectar quality (Baker & Baker 1983) as well as the mouthpart structure of the agent (Jervis 1998; Baggen *et al.* 1999) are likely to influence the suitability of flowering plants. A logical hierarchy for measuring the success of CBC in the field, after selection of appropriate floral resources is (modified from (Wratten *et al.* 2003a)):

1. Do the adult parasitoids aggregate near floral resources in the field?
2. Do the parasitoids use any resource subsidies such as pollen and nectar?
3. Is the fitness of parasitoids improved by provision of floral resources?
4. Are parasitism rates increased by provision of floral resources?
5. Are pest populations reduced?
6. Are pest populations reduced to below economic thresholds?

This research programme aims to address the first four questions, while questions five and six are beyond the scope of this research.

1.8 Objectives

The aim of this research is to identify and provide suitable floral resources to enhance the efficacy of *M. hyperodae* without benefiting *L. bonariensis*. It is beyond the scope of this research to develop a commercial seed mixture to sow in grasslands to provide floral resources to *M. hyperodae*. The objectives of this research were:

1. To identify the most suitable floral resource to increase the longevity of *M. hyperodae* (Chapter 2).
2. To study lifetime carbohydrate reserves in fed and unfed *M. hyperodae* (Chapter 3).
3. To measure the extent of aggregation of parasitoids at floral resources (Chapter 4).
4. To determine the extent of floral resource use by parasitoids in the field (Chapter 5).
5. To compare parasitism rates of *L. bonariensis* in pasture with and without floral resources (Chapter 6).

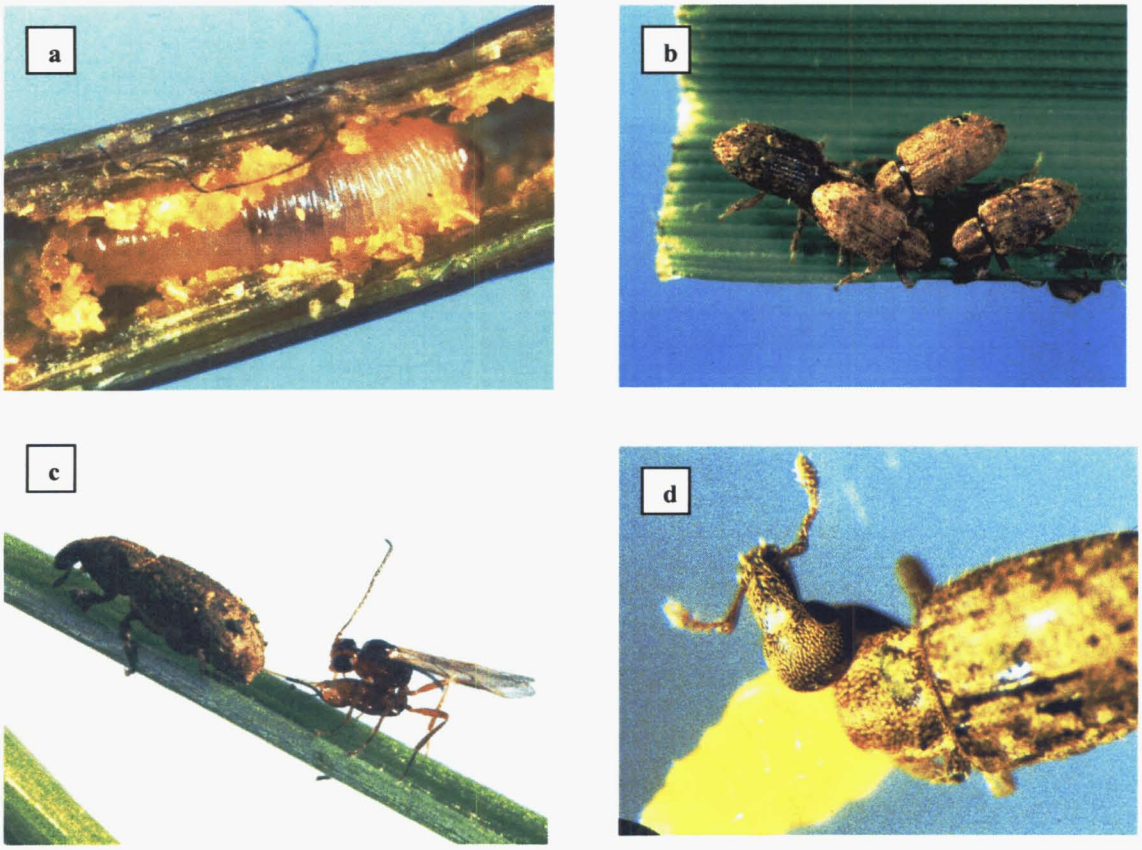

Plate 1.1: a) *Listronotus bonariensis* larva. The rye grass tiller was cut longitudinally to expose the larva, b) *L. bonariensis* feeding on ryegrass leaf, c) *Microctonus hyperodae* ovipositing on *L. bonariensis* and. d) *M. hyperodae* larva emerging from *L. bonariensis*

(Photos: AgResearch)

CHAPTER 2

THE EFFECTS OF FLOWER MORPHOLOGY AND NECTAR QUALITY ON THE LONGEVITY OF *Microctonus hyperodae*

2.1 Introduction

Many species of predatory and parasitic insects exploit non-host foods (Landis *et al.* 2000; Gurr *et al.* 2004) such as nectar (Takasu & Lewis 1996; Baggen & Gurr 1998). Numerous parasitoid species have been reported feeding on a broad range of flowers (Jervis *et al.* 1993). Floral nectar contains sugars, proteins, amino acids, lipids and many other organic and inorganic substances (Hagen 1986). Access to nectar can substantially increase the longevity and fecundity of natural enemies (Shahjahan 1974; Baggen & Gurr 1998; Berndt *et al.* 2002; Irvin *et al.* in press). Nectar is vital for egg maturation in some insects (Jervis & Kidd 1996) and can more than double prey/host mortality rates (White *et al.* 1995). Provision of suitable non-host foods, therefore, offers opportunities to increase natural enemies' fitness and enhance biological control (Jervis *et al.* 1993).

Natural enemy species exhibit interspecific variation in their preferences for, and responses to, different flower species, but the mechanisms underlying this variation remain poorly understood (Jervis & Kidd 1986; Patt *et al.* 1997). Floral architecture is likely to be one of the factors which govern the exploitation of flowers by insect natural enemies (Patt *et al.* 1997), since it has already been recognised as an important determinant of pollination by insects (Kevan & Baker 1983). Mouthpart structure and parasitoid size play important roles in access to nectar sources (Patt *et al.* 1997; Jervis 1998). Nectar quality is also likely to influence the effects of flowers on parasitoids. Baker and Baker (1983) stated that parasitoids prefer sucrose-dominant flower nectar. They analysed nectar from 765 species and suggested a classification system based on the sucrose/hexose (mainly glucose and fructose) ratio of nectars. This classification had four ratio classes: hexose-rich (<0.1), hexose-dominant (0.1-0.49), sucrose-rich (0.5-0.99) and sucrose-dominant (>0.99). The glucose/fructose ratio, the ratio of the two major hexose sugars present in all nectar sources, was nearly one and this was consistent across all flower species tested.

Selecting and providing flower species which can be accessed by, and are beneficial to, natural enemies is central to the concept of enhancing biological control by habitat manipulation. This might be achieved by providing *M. hyperodae* adults with appropriate food since, under laboratory conditions, the longevity of *M. hyperodae* has been significantly increased by the provision of either glucose or honey solutions (Hodgson *et al.* 1993; Phillips 1998). Moreover, many experiments show that parasitoids' longevity and fecundity can be increased by providing them with sugar sources such as sucrose and honey solutions as well as by providing nectar producing flowers in the laboratory conditions (Table 2.1)

The possibility of increasing both longevity and fecundity is dependent on parasitoid ovigeny. The 'ovigeny' index, introduced by Jervis *et al.* (2001), defines *M. hyperodae* as a synovogenic species because maturation of some eggs occurs just after adult eclosion (Phillips & Barid 2001). However *M. hyperodae* emerges with its final egg load and produces no eggs during its life span (Phillips 1998; Phillips & Baird 2001). According to an earlier classification, provided by Flanders (1950), *M. hyperodae* can also be considered as a pro-ovigenic species. It is, therefore, more sensible to use the later definition since egg storage capacity of *M. hyperodae* equates to the maximum potential reproductive success (Heimpel & Rosenheim 1998). Moreover, *M. hyperodae* does not require any food supplement for egg maturation. Therefore, for the purpose of this study *M. hyperodae* can be regarded as a pro-ovigenic parasitoid. It is almost certain that the provision of sugar sources can increase the longevity of *M. hyperodae*, but not the fecundity (Phillips & Baird 2001).

Table 2.1: Summary of selected studies that show honey, sugar solutions, flowering plants and honeydew increased longevity (L) and/or fecundity (F) of different parasitoids.

Family	Species	Results	Reference	
Braconidae	*Costesia glomerata*	sucrose, glucose, fructose increased L	Wäckers (2001)	Lab. experiment
	Costesia rubecula	sugar solution increased L by factor of 9-14	Wäckers & Swaan (1993)	Lab. experiment
	Coeloides bostrychorum	various flowers increased L	Hougardy & Grégoire (2000)	Lab. experiment
	Dolichogenidea tasmanica	alyssum flower increased L by factor of 9	Berndt & Wratten (2005)	Lab. experiment

	Heterospilus prosopidis	honey increased L by factor 5-7	Wäckers (1998)	Lab. experiment
	Microctonus hyperodae	glucose increased L by factor 1.5-2.1 and honey increased L by factor of 4	Hodgson *et al.* (1993) and Phillips (1998)	Lab. experiments
	Peristenus pseudopallipes	flowers of *Erigeron canadensis, E. strigosus* and *Daucus carota* increased L	Shahjahan (1974)	Lab. Experiment
Ichneu-monidae	*Diadegma semiclausum*	buckwheat flowers increased L and F	Lavandero *et al.* (in press-a)	Lab. Experiment
Trichogrammatidae	*Trichogramma brassicae*	honey increased L	Gurr & Nicol (2000)	Lab. Experiment
	Trichogramma carverae	honey increased L	Gurr & Nicol (2000)	Lab. Experiment
	Trichogramma minutum	honey increased L and F	Leatemia *et al.* (1995)	Lab. Experiment
	Trichogramma nubilale	honey increased L and F	Olson & Andow (1998)	Lab. Experiment
	Trichogramma platneri	honey, sucrose and fructose increased L	McDougall (1997)	Lab. and field experiment

In the present work, the effects of seven flower species , a 50% honey solution and water on the longevity of *M. hyperodae* were evaluated in the laboratory. In addition, variables relating to flower morphology and nectar quality were measured to identify possible explanations for differences in longevity between treatments.

2.2 Materials and methods

2.2.1 Insects

Adult *L. bonariensis* were collected by sweeping grass at night, either at the Lincoln University Dairy Farm, or on roadside verges near Lincoln. The weevils were maintained in the laboratory at 20°C (±2°C) with a 16:8h (L:D) photoperiod to allow parasitoid larvae to emerge and develop to the pupal stage. Rearing cages were checked for pupal cocoons every 24h (see Goldson *et al.* (1993) for rearing methods). Cocoons were transferred to Petri dishes,

each containing a moistened cotton bud to maintain humidity, and kept under the conditions noted above. The dishes were checked every 24h for newly emerged parasitoids.

2.2.2 Experimental design

Selection of flowering plants for this experiment was based on two criteria. First, the proven ability of those flower species to increase longevity or fecundity or both these aspects of parasitoid's biology, and, second, plants that are agronomically compatible with pastures.

Two separate laboratory experiments were conducted so that flowering could be synchronised with the emergence of adult parasitoids. In the first experiment, Phacelia (*Phacelia tanacetifolia* Bentham cv. Balo (Hydrophyllaceae)), buckwheat (*Fagopyrum esculentum* Moench cv. Katowase (Polygonaceae)), alyssum (*Lobularia maritima* L. cv. Carpet of Snow (Brassicaceae)), a 50% honey solution and water were tested using six replicates blocked over time. In the second experiment, coriander (*Coriandrum sativum* L cv. Slowbolt (Apiaceae)), white clover (*Trifolium repens* cv. Grassland Kopu (Fabaceae)), red clover (*Trifolium pratense* cv. Astred (Fabaceae)), white mustard (*Sinapis alba* cv. Emego (Brassicaceae)), buckwheat and water were tested, also using six replicates blocked over time. The buckwheat and water treatments were, therefore, common to both experiments. All plants were grown to the flowering stage under greenhouse conditions (18°C ± 4°C and 16:8h L:D).

The experimental cages consisted of cylindrical containers made from acetate sheets (20 x 10cm). The container top was covered with a cloth mesh to provide ventilation. Circular plugs were cut to fit the bottom of the container and these had a central slit to enable flowering plant shoots to be introduced to the cage from the plant pot. Each container was attached to a wooden rod with a rubber band and the wooden rod was inserted into the pot soil. Parasitoids were introduced through a small hole in the side of the container, which was subsequently plugged with cotton wool. Similar cages were used for the 50% honey and water treatments, except that these two treatments were provided in a 40mm vial sealed with a cotton wool plug. Each vial was centrally placed in the bottom of the cage. The *M. hyperodae* adults used had eclosed less than 24h beforehand, and one was used per replicate. *M. hyperodae* had constant access to treatments. Survival was recorded every 24h until the last parasitoid had died.

2.2.3 Measurements of parasitoid and flower morphology

All dead *M. hyperodae* were frozen for subsequent measurement of the length of the hind tibia (Phillips & Baird 2001) and the width of the parasitoid head at 50X magnification using an ocular micrometer (Graticules Ltd) fitted to a microscope following Baggen *et al.* (1999) and Winkler *et al.* (2003). The head measurement was taken between the extreme margins of each eye; corolla depth and corolla opening width (aperture) measurement of the flowers used for this experiment were made using the same approach.

2.2.4 Nectar quality measurements

Floral nectar was collected from buckwheat, coriander, white mustard and phacelia flowers between 09.00h and 10.00h following the suggestions made by Lee & Heimpel (2003). Four samples were collected from each of four different plants of each species using micro capillary tubes under a microscope at 50X magnification. A microelectrode puller (Narishige, model pp-830) was used to make the tubes. The nectar collected from each plant was immediately transferred into a 0.6ml Eppendorf tube that contained 70% ethanol to stop enzyme activity. All sealed samples were then taken to the Netherlands Institute for Terrestrial Ecology for analysis using High Performance Liquid Chromatography (HPLC). After diluting (ethanol to 8%) 10µl of each nectar sample was injected into a Dionex DX 500 HPLC system (Dionex Corp., Sunnyvale, CA), equipped with a GP 40 gradient pump, a Carbopac PA1 guard (4 x 50 mm), an analytical column (4 x 250 mm) and an ED 40 Electrochemical Detector for Pulsed Amperimetric Detection (PAD). The column was eluted with 1 M NaOH and Milli-Q water (1: 9, 1ml m^{-1}) and kept at 20°C during analysis. Reference curves were obtained for sorbitol, manitol, trehalose, glucose, fructose, melibiose, sucrose, melezitose, raffinose, maltose and erlose by injecting calibration standards with concentrations of 2.5ppm, 5ppm, 7.5ppm and 10ppm. The correlation coefficient (r^2) for the calibration lines was always higher than 0.95. Individual nectar samples were analysed using the program PEAKNET software release 5.1 (DX-LAN module).

2.2.5 Statistical analysis

Survival analysis (SAS V.8) was used to compare the effect of the different plant nectars on parasitoid longevity. The Kaplan-Meier survival function was determined and survival curves compared using Cox's Proportional Hazards Model (Afifi & Clark 1990; Hosmer & Lemeshow 1999).

2.3 Results

2.3.1 Longevity of *M. hyperodae*

In both experiments, the highest mean longevity for *M. hyperodae* was obtained from buckwheat. The minimum longevity over all replicates for buckwheat was 15 days. In contrast, the maximum longevity of *M. hyperodae* in all other treatments was less than 15 days, except for two replicates in the honey treatment. There was no significant relationship between total body sugar levels and tibia length ($P > 0.05$, $r^2 = 0.13$ and $n = 66$). So, parasitoid body size was not considered in subsequent analysis.

There were also significant differences between alyssum and phacelia, water and phacelia, and water and honey (Figure 2.1a). However, there was no difference between alyssum and water. It is notable that longevity in the water treatment was significantly higher than that with phacelia. Figure 2.1b shows the survival distribution functions from the first laboratory experiment. Paired comparisons between the different treatments using Cox's Regression Model showed that there was a significant difference between buckwheat and all other treatments ($P < 0.01$).

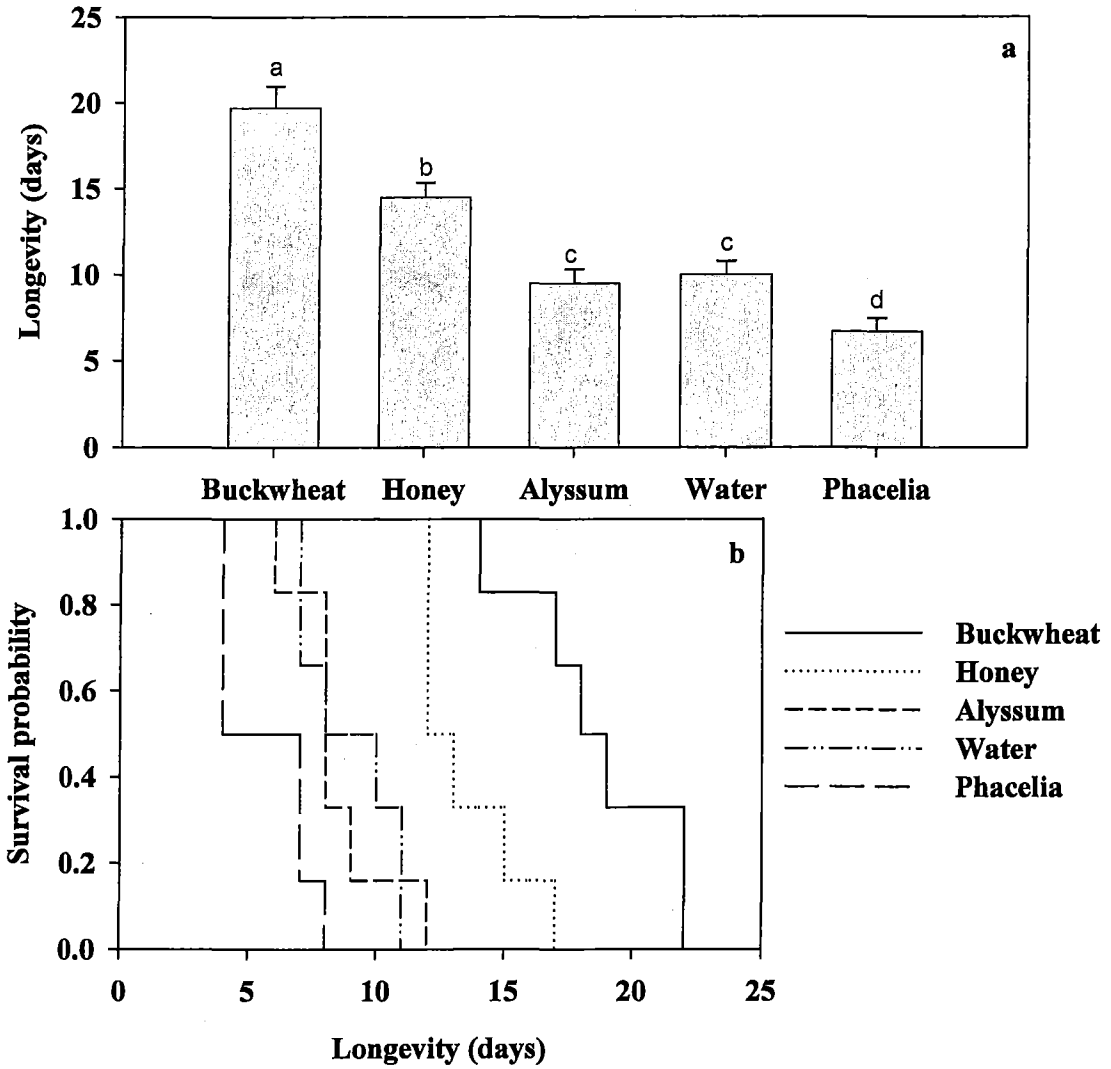

Figure 2.1: *a) M. hyperodae*'s longevity with three flowering plants, a 50% honey solution and the control treatment water. Bars sharing the same letter do not differ at $P = 0.05$, error bars = +SE, n = 6. b) Kaplan-Meier estimates of the survival functions of *M. hyperodae*.

There were significant differences between buckwheat and coriander compared with water (Figure 2.2a). Figure 2.2b shows the survival functions from the second laboratory experiment Paired comparisons between the different treatments using Cox's Regression Model showed that there was a significant difference between buckwheat and all other treatments ($P < 0.01$). There were also significant differences between coriander and white mustard, coriander and water, coriander and two clover species, white mustard and two clover species water and two clover species. However, there was no difference between white mustard and water.

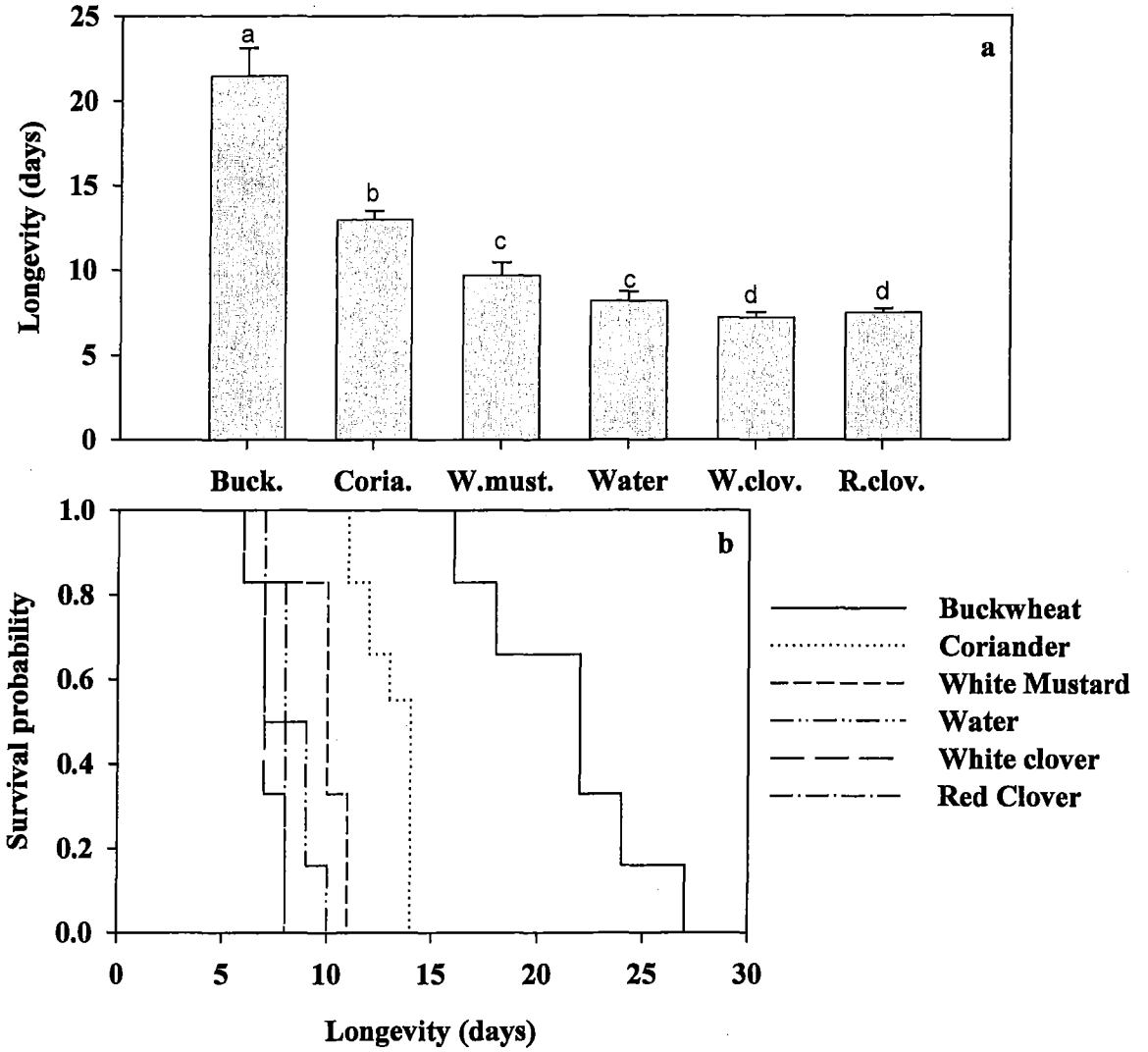

Figure 2.2: a) *M. hyperodae*'s longevity with five flowering plants, and the control treatment water. Bars sharing the same letter do not differ at P = 0.05, error bars = +SE, n = 6. b) Kaplan-Meier estimates of the survival functions of *M. hyperodae*.

2.3.2 Flower architecture and parasitoid head width measurements

Corolla depths ranged from a minimum of 0 mm to a maximum of 11.3 ± 0.41mm (± SE). Coriander had the shallowest corolla, followed by buckwheat, alyssum, white mustard, phacelia, white clover and red clover (Fig. 2.3). Corolla aperture widths ranged from a maximum of 6.8 ± 0.22mm to a minimum of 0.19 ± 0.13mm. Buckwheat had the widest corolla aperture, followed by phacelia, white mustard, alyssum, coriander, red clover and white clover (Fig. 2.3). *M. hyperodae* females had a mean head width of 0.32 ± 0.17mm.

2.3.3 Nectar quality

Nectar from only phacelia, buckwheat, coriander and white mustard was analysed to obtain sugar profiles because preliminary observations suggested that flower morphology in relation to the parasitoid's head width prevented access to the two clover species (see section 2.3.2) and to alyssum (see section 2.4 for more details). The nectar quality results obtained from HPLC analysis showed that the glucose/fructose ratio was similar for all nectars (Figure. 2.4). However, the sucrose/(glucose + fructose) ratio showed marked differences between plant species. Buckwheat had the highest ratio of 1.44 while ratios for phacelia, coriander and white mustard were 1.12, 0.10 and 0.01 respectively.

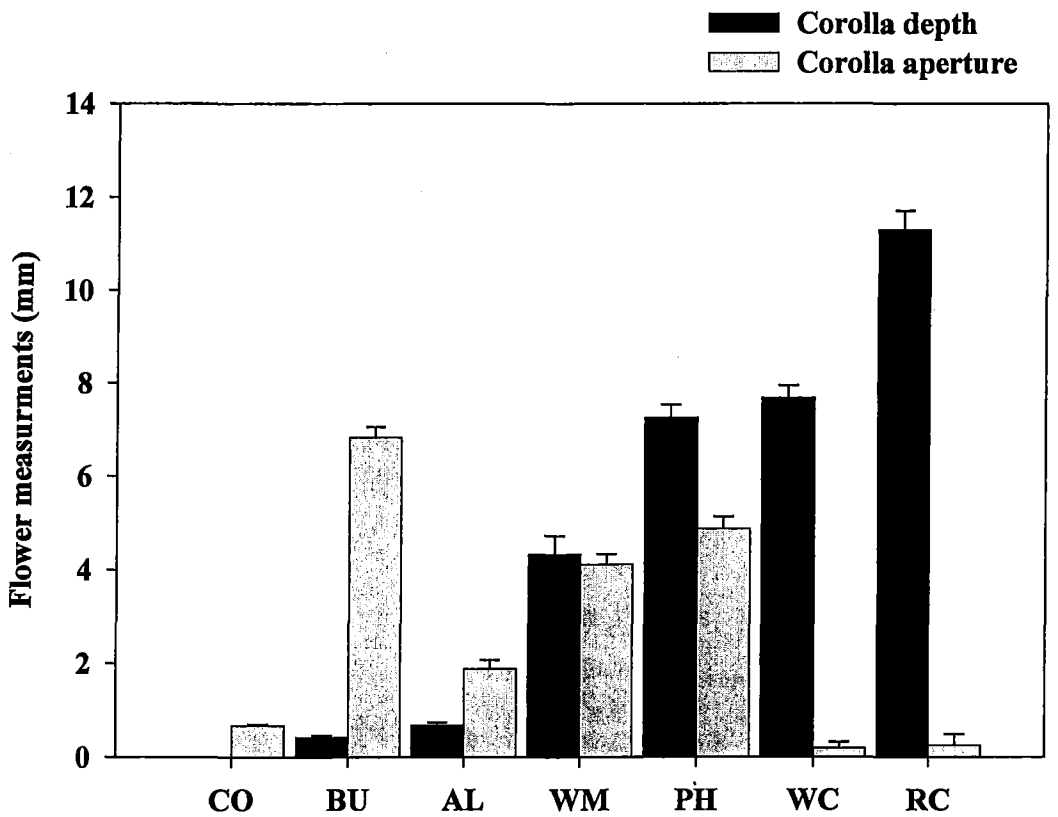

Figure 2. 3: Floral architecture measurements of flowering plants, error bars = +SE, n = 15 flowers per flowering plant. CO: coriander, BU: buckwheat, AL: alyssum, WM: white mustard, PH: phacelia, WC: white clover, RC: red clover.

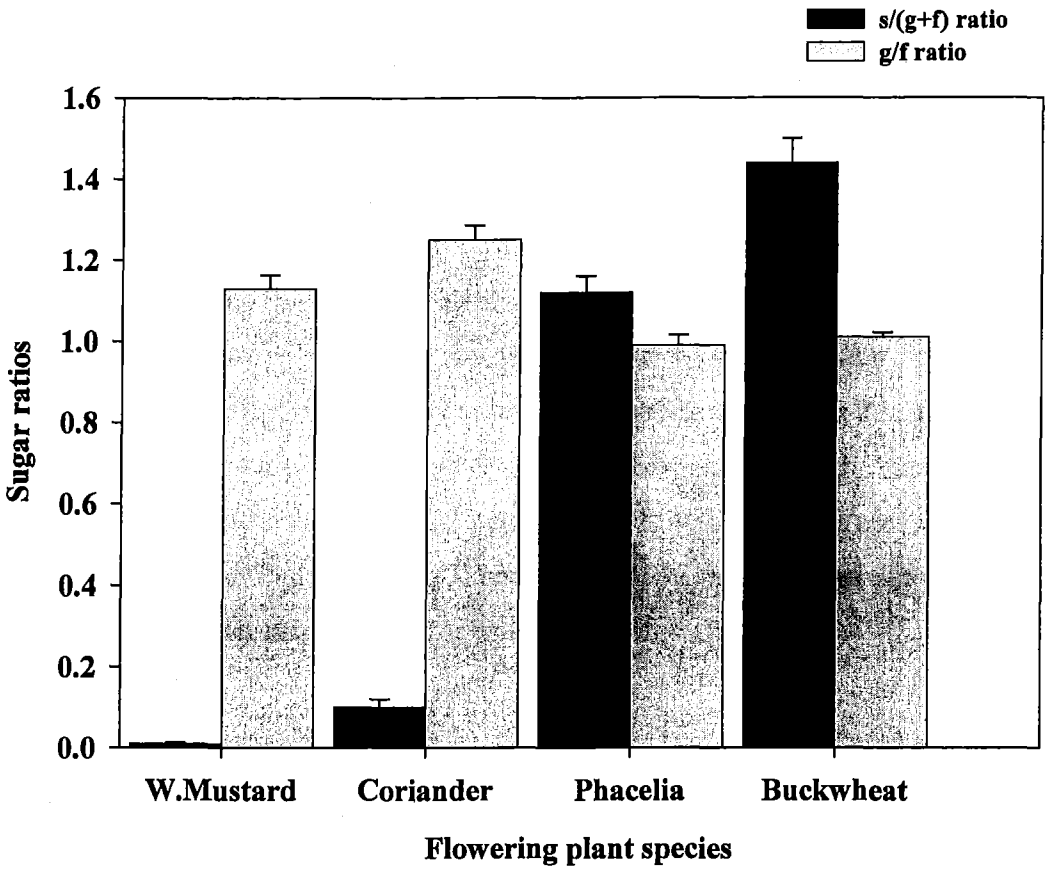

Figure 2.4: Two sugar ratios calculated from floral nectar analysis, error bars = +SE, n = 4 nectar samples per species (Four samples were taken from four different plants). The ratios were glucose/fructose (g/f) and sucrose/(glucose+fructose) [s/(g+f)].

2.4 Discussion

2.4.1 Benefits from floral resources

Of the seven flower species tested, only buckwheat, coriander and white mustard provided greater benefits to *M. hyperodae* longevity than water (although only buckwheat and coriander gave a significant improvement). Of these three most beneficial flowers, buckwheat significantly increased *M. hyperodae* longevity compared with all other treatments, including the second-best treatment, the 50% honey solution.

2.4.2 The role of floral architecture in increasing the longevity of *M. hyperodae*

Comparisons of the mean head width of *M. hyperodae* with the corolla aperture measurements shown in Figure 2.3 suggested that *M. hyperodae* should have been able to access the nectar of all of the flowers tested, with the exceptions of white clover and red clover. In the latter two cases, the corolla aperture appeared too narrow for *M. hyperodae* to insert its head, and this may explain why these flowers did not increase *M. hyperodae* longevity. It is also possible that the deep corollae of these flowers impeded access by *M. hyperodae*. This is unfortunate since clovers are abundant in New Zealand pastures and may otherwise have provided a food source for *M. hyperodae* adults. In the case of alyssum, the small gaps between the stamens and petals of the flower probably prevented *M. hyperodae*'s access to the nectar. Patt *et al.* (1997) and Jervis (1998) also pointed out that some parasitoids cannot access the alyssum flower due to this limitation (Plate 2.1).

Of the more beneficial flowers, buckwheat and white mustard were characterised by having corollas which were of intermediate or shallow depth, and had broad apertures. Coriander was unusual in that its corolla had a relatively narrow aperture which was similar in width to *M. hyperodae*'s head, but this possible limitation to access by *M. hyperodae* would have been completely negated by coriander's extremely shallow corolla. While all three flower species, therefore, appeared to provide *M. hyperodae* with ready access to nectar, only buckwheat provided a substantial increase in parasitoid longevity. *M. hyperodae* does not have any mouthparts that are specialised for feeding from flowers such as clover species.

2.4.3 The role of nectar quality in increasing the longevity of *M. hyperodae*

The nectar quality measurements provided a possible reason for the difference between buckwheat and coriander that could not be explained by their floral architectures. Buckwheat had the highest s/(g+f) ratio, followed by coriander and white mustard. Using the classification of Baker and Baker (1983), buckwheat falls into the sucrose-dominant class, whereas coriander is hexose-rich and white mustard is hexose-dominant. It has been suggested that, as pollinating insects consume nectar, their osmotic pressure increases, (Watt *et al.* 1974; Baker & Baker 1983), and such physiological constraints can limit dietary selection by insects (Bluthgen & Fiedler 2004). This increase in pollinators' osmotic pressure occurred more rapidly when they consumed nectar that contained a high proportion of monosaccharides such as glucose or fructose, rather than disaccharides such as sucrose,

because the number of molecules is higher in the former case (Watt *et al.* 1974; Baker & Baker 1983). Due to this osmotic effect it is, therefore, possible that *M. hyperodae* was able to consume greater quantities of high-disaccharide nectar (e.g., buckwheat) than high-monosaccharide nectar (e.g., white mustard and coriander), and thereby derive greater benefits from buckwheat.

2.4.4 The role of the architecture of phacelia flower

Perhaps the most unexpected result arose from phacelia, which conferred the shortest longevity to *M. hyperodae*, as phacelia has previously been shown to benefit other insect natural enemies, both in the laboratory and in the field (Holland *et al.* 1994; White *et al.* 1995; Hickman & Wratten 1996; Baggen *et al.* 1999; Gurr *et al.* 2000b). Moreover, in this study phacelia nectar exhibited a s/(g+f) ratio second only to buckwheat. Although phacelia has a corolla aperture broad enough for *M. hyperodae* to insert its head, the corolla is also relatively deep, and it is uncertain if *M. hyperodae* will enter relatively deep corollae to access nectar, or if it restricts its foraging to flowers with corollae shallow enough for it to remain on the flower surface as it feeds. Phacelia flowers have stamen appendages and erect hairs, which is a characteristic of the family Hydrophyllaceae (Plate 2.1b). These appendages have been reported to limit the access of other parasitoids to flower nectarines (Lavandero *et al.* in press) In addition, it has been reported that *Dolichogenidae tasmanica* (Cameron) (Hymenoptera: Braconidae) could not access phacelia flowers (Irvin *et al.* in press). The most likely explanations for this result are that 1) *M. hyperodae* was unable to access phacelia nectar and, in the absence of accessible moisture in the experimental cages, *M. hyperodae* longevity was reduced relative to the water control and 2) *M. hyperodae* was deterred by volatiles from the phacelia plant.

2.4.5 Relevance of these findings in selecting floral resources

Parasitoids' access to nectar is predominantly influenced by the morphology of the flowers (Patt *et al.* 1997; Jervis 1998; Wäckers 2004) and nectar quality (Baker & Baker 1983), as well as the mouthparts of the parasitoid (Jervis 1998; Baggen *et al.* 1999). This study has provided evidence that *M. hyperodae* prefers to feed on relatively open nectar sources, as do many other hymenopterans parasitoids that have generalised mouth parts (Gilbert 1998). This work has also indicated that parasitoid head-width and the sucrose/(glucose+fructose) ratio in

the nectar may be important factors to consider when choosing non-host foods for natural enemies. This information will be of use in adding the most appropriate floral diversity to agro-ecosystems via 'ecological engineering' Gurr *et al.* (2004) to improve the effectiveness of biological control.

2.5 Conclusions

The nectar quality measurements provided an expainable reason for the difference between buckwheat and coriander that could not be explained by their floral measurements. The findings from this study allow us almost certainly to rule out the two clover species, and phacelia and alyssum based on their floral architecture measurements. It is possible that parasitoids could access clover nectar from bee tripped or damaged flowers in the field. However some parasitoids tested against phacelia and alyssum in other studies showed promising results in increasing their 'fitness' (Table 2.1 and section 2.4.4). It would be interesting to explain these findings by testing the hypotheses that a) *M. hyperodae* is deterred by volatiles from these plant species or b) it could not benefit from these plants due to its inability to reach the nectaries. Visual recording of behaviour studies with different flower species could provide some important information in this regard.

It is also important to test whether *M. hyperodae* will benefit by provision of buckwheat plants in the field. Field situations are very different from laboratory conditions and it may be possible that they do not get the same benefit from buckwheat as they did in the laboratory. Field testing of *M. hyperodae*'s response to buckwheat in the field is presented in Chapter 4.

Plate 2.1: Close up views of (a) alyssum flowers show small gaps between the stamens and petals and (b) phacelia flower shows stamen appendages and erect hairs.

CHAPTER 3

EFFECTS OF SUGAR FEEDING ON LIFETIME CARBOHYDRATE RESERVES AND THEIR RELATION TO NUTRITIONAL STATE OF *Microctonus hyperodae*

3.1 Introduction

A field study of *M. hyperodae* and *L. bonariensis* population dynamics showed that adult parasitoids are abundant, but hosts are scarce, during early summer (Phillips *et al.* 1998). Analysis indicated that enhancing *M. hyperodae* longevity during this period, perhaps through provision of nectar sources, could increase parasitism of *L. bonariensis* (Phillips *et al.* 1998). Previous studies have shown that the longevity of *M. hyperodae* adults can be significantly increased by provision of either glucose or honey solutions (Hodgson *et al.* 1993; Phillips 1998). Parasitoids provided with buckwheat and coriander had significantly increased longevity in the laboratory (Chapter 2). This chapter focuses on the measurement of carbohydrate reserves throughout the adult life spans of fed and unfed *M. hyperodae*.

Carbohydrates are the main energy source for adult parasitoids, which feed on several different sugar sources, such as floral and extrafloral nectar, and aphid honeydew (Jervis *et al.* 1993). If the energy reserves that parasitoid adults carry over from their larval stage are not supplemented by additional sugar sources, then aspects of their potential fitness could be reduced. Many laboratory experiments have shown that sugars can increase the longevity and fecundity of parasitoids (see Chapter 2 for details).

Insects use stored fats and carbohydrates as sources of energy. Parasitoid adults with access to supplementary food have increased sugar and glycogen reserves, whereas both types of reserves rapidly decline in unfed parasitoids. Fat reserves decline continuously in fed and unfed parasitoids, but this depletion occurs rapidly in the unfed ones, and only gradually in the fed parasitoids (Ellers 1996; Olson *et al.* 2000; Giron & Casas 2003). Lipogenesis may not occur at all in parasitoids (Ellers 1996; Olson *et al.* 2000; Giron & Casas 2003), thus fat levels are probably unreliable for indicating whether or not parasitoid adults have exploited supplementary

food, while carbohydrates levels are much more likely to be useful in this way (Wäckers & Steppuhn 2003).

Several studies have examined changes in nutrient levels during the lifetimes of parasitoid adults. Olson *et al.* (2000) used the anthrone test (van Handel 1967) to quantify changes in the levels of fructose, glycogen and total sugars in fed and unfed parasitoids. Wäckers & Steppuhn (2003) and Steppuhn & Wäckers (2004) developed a more sensitive method using high performance liquid chromatography (HPLC) to identify and quantify a range of sugars in individual parasitoids. These studies showed that fed parasitoids had higher sugar levels than unfed ones, at least for the initial part of their adult life, and that they lived longer.

Unfed *M. hyperodae* adults lived up to 10 days with water in the laboratory (Hodgson *et al.* 1993; Goldson *et al.* 1995; Phillips 1998; Vattala *et al.* 2004) which was an unexpectedly long time because many other parasitoids survive only 2-3 days without sugar (Siekmann *et al.* 2001; Scarratt 2002; Steppuhn & Wäckers 2004; Lavandero *et al.* in press-a). More recent laboratory experiments have shown both that *M. hyperodae* will feed on flowers and that floral nectar can increase its longevity to 21 days (Chapter 2).

To exploit opportunities to improve biological control, it is important to gain a better understanding of how sugar sources influence the energy reserves of *M. hyperodae* adults. Accordingly, this chapter addresses the following questions: What is the mean body-sugar level in recently-eclosed *M. hyperodae* adults and how does this change over time if they do not have access to sugar? What are the effects of sugar feeding on *M. hyperodae* body sugar levels? What changes occur in body sugar levels of parasitoids starved for 12 hours after feeding from buckwheat, and is it possible to detect any difference in sugar composition in honey-fed and buckwheat-fed parasitoids?

3.2 Materials and methods

3.2.1 Insects

Adult *L. bonariensis* were collected by sweeping grasses at night, either at the Lincoln University Dairy Farm, or from roadside verges near Lincoln. Weevils were maintained in the laboratory at 20°C (±2°C) with a 16:8 h (L:D) photoperiod to allow parasitoid larvae to emerge and pupate. Rearing methods for parasitoids followed those of Goldson *et al.* (1993). Cages were checked for cocoons every 24h and all cocoons were transferred to Petri dishes that were kept under the above conditions. A cotton bud soaked in water was placed in each Petri dish to maintain high humidity. The dishes were checked every 24h for newly emerged parasitoids.

3.2.2 Experimental design

Continously fed and unfed parasitoids

Newly emerged parasitoid adults were maintained individually in separate Petri dishes. The two treatments involved providing parasitoid adults with *ad libitum* access to either 50% honey solution, or water. These liquids were made available on a piece of cotton wool placed in each dish, and were replenished every two days. As parasitoid adults emerged, they were randomly assigned to a treatment. A sub-sample of parasitoids from the honey treatment was frozen daily at -80°C, from age 1 to 9 days and every second day thereafter, up to the maximum longevity of 19 days. In the water treatment, a sub-sample of parasitoids was frozen at -80°C daily from age 1 to the maximum longevity of 8 days. Parasitoids lived up to 19 and 8 days with honey and water, respectively, in this experiment. The number of replicates for each age category ranged from 3 to 10 parasitoids per treatment (Table 3.1). In addition, twelve recently emerged parasitoids were not subjected to any experimental treatment, but were frozen at -80°C for subsequent analysis of sugar levels.

Table 3.1: The number of replicates used for HPLC analysis in laboratory reared *M. hyperodae*.

Treatment	Days after emergence													
	1	2	3	4	5	6	7	8	9	11	13	15	17	19
Newly emerged	12													
Unfed		6	6	3	3	3	3	3						
Fed		7	7	8	4	4	5	3	3	5	5	4	3	3

Parasitoids starved after having fed on buckwheat

Experimental cages that consisted of cylindrical containers made from acetate sheets (as used for the plant screening experiment in Chapter 2) were used to confine parasitoids on the flowering buckwheat. The *M. hyperodae* adults used had eclosed less than 24h beforehand, and one parasitoid was used per cage. They were left on buckwheat for two days (48h) before being transferred to another empty cage where they were left to starve for different intervals (Table 3.2) before freezing at -80°C. Table 3.2 shows the number of parasitoids used to analyse body sugars from 0 to 12h. Before transferring all parasitoids from the freezer to 70% ethanol for analysis of sugar levels, their size was estimated by measuring the length of the hind tibia following Phillips & Baird (2001).

Table 3.2: The number of parasitoids used for HPLC analysis from 0 to 12 hours after having fed on buckwheat.

	Time after feeding (h)								
	0	0.5	1	2	3	4	5	6	12
Parasitoid number	3	3	3	3	3	3	3	3	3

3.2.3 Parasitoid sugar analysis using HPLC

For sugar analysis, each insect was transferred to 1ml of 70% ethanol and stored at room temperature in a 5ml Eppendorf tube. The parasitoid was crushed using a pestle inside the tube, centrifuged at 13000rpm for ten minutes, then 500µl of the supernatant was diluted ten-fold with Milli-Q water. Ten µl of each sample were injected into a Dionex DX 500 HPLC system (Dionex Corp., Sunnyvale, CA), equipped with a GP 40 gradient pump, a Carbopac PA1 guard (4 x 50

mm), an analytical column (4 x 250 mm) and an ED 40 Electrochemical Detector for Pulsed Amperimetric Detection (PAD). The column was eluted with 1M NaOH and Milli-Q water (1: 9, 1 ml m^{-1}) and kept at 20°C during analysis. Reference curves were obtained for sorbitol, manitol, trehalose, glucose, fructose, melibiose, sucrose, melezitose, raffinose, maltose and erlose by injecting calibration standards with concentrations of 2.5 ppm, 5 ppm, 7.5 ppm and 10 ppm of these sugars. The correlation coefficient (r^2) for the calibration lines was always higher than 0.95. Individual sugar concentrations of parasitoids were analysed using the program PEAKNET software release 5.1 (DX-LAN module).

3.2.4 Statistical analysis

GenStat Version 8 was used for sugar analysis. The data showed lack of normality so non-parametric statistics were used. The sugar concentrations for parasitoids from different treatments and ages were compared pair-wise using a Mann-Whitney U-test. When testing for differences between ages, Kruskal-Wallis ANOVA was used.

3.3 Results

3.3.1 Sugar levels in newly emerged parasitoids

The mean total sugar level of newly emerged parasitoids was 17.2 ± 2.55µg (± SE; n = 12). Sorbitol, glucose, fructose, sucrose and maltose were recorded from newly emerged parasitoids. The major fraction of total sugars of newly emerged parasitoids was glucose (60%), followed by maltose (16%), fructose (12%), sorbitol (5%) and sucrose (4%). The common insect haemolymph sugar, trehalose, was not detected in newly emerged parasitoids.

3.3.2 Sugar levels in unfed parasitoids

Total sugar levels gradually decreased with age in unfed parasitoids (Figure 3.1). They had declined to 16.2 ± 3.65µg (n = 6) on the second day and to 13.1 ± 2.16µg (n = 6) on the third day.

These values, as a percentage of initial total sugar levels, were 94% and 76%, respectively. On the eighth day, they had declined to 15%. Glucose was the main sugar detected in unfed parasitoids. Trehalose was never detected in unfed parasitoids.

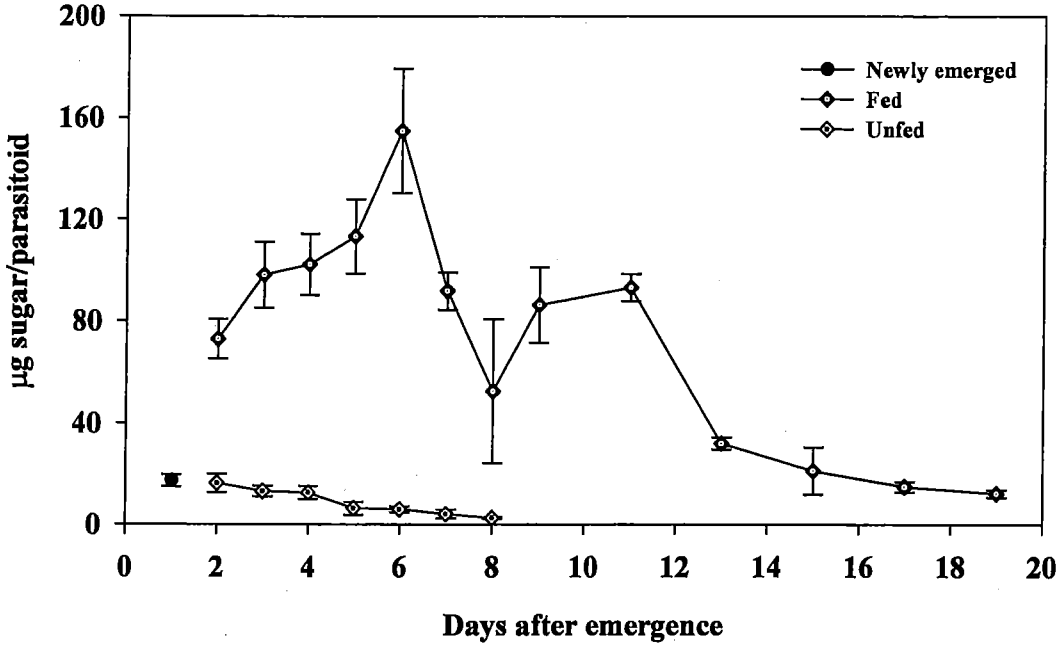

Figure 3.1: Comparison of *Microctonus hyperodae* adult mean total sugar levels (µg/parasitoid) in newly emerged adults, unfed adults (day 2-8) and fed adults (day 2 -19). Error bars = ±SE.

Table 3.3 shows the sugar level changes over the lifespan of *M. hyperodae*. The total sugar levels in unfed parasitoids were always lower than those of newly emerged parasitoids. In fed parasitoids, the total sugar levels remained high from day two to day 15, compared with both newly emerged and unfed parasitoids. Total sugar levels were lower on day 17 and 19 in fed parasitoids than those in newly emerged and unfed insects on day two (Table 3.3) .

Table 3.3: Mean total sugar levels in newly emerged, fed and unfed parasitoids. Shaded areas highlight similar total sugar levels in different treatments.

	Total sugars µg/parasitoid		
Days	Newly emerged	Unfed	Fed
1	*17.2*		
2		*16.2*	72.9
3		*13.1*	97.9
4		*12.5*	102.1
5		6.3	113.1
6		5.9	154.7
7		4.0	91.7
8		2.5	52.5
9			86.2
11			93.0
13			32.0
15			21.2
17			*14.8*
19			*12.2*

3.3.3 Sugar levels in continuously-fed parasitoids

Honey feeding significantly increased the total sugar levels of *M. hyperodae* (Figure 3.1). On day two, after feeding for 24h, sugar levels increased to 72.1 ± 7.68µg (n = 7) per parasitoid, and on day six these reached their maximum of 154.7 ± 24.46µg (n = 5) per parasitoid. Total sugar levels then gradually declined, and reached 12.1 ± 1.47µg (n = 3) per parasitoid on the 19th day. There was a significant difference in sugar composition between fed and unfed parasitoids ($P < 0.01$). Fructose levels were significantly higher in fed parasitoids and levels increased immediately after feeding and, thereafter, remained approximately equal to those of glucose throughout the insects' lifespans (Figure 3.2). The common insect haemolymph sugar, trehalose, was detected in *M. hyperodae* after feeding for 24h. Trehalose was continuously present until day 13, but was not subsequently detected. However, trehalose levels were low compared with other sugars and the maximum recorded on day nine was 1.4 ± 0.37µg (n = 3) per parasitoid. Comparatively low levels of sucrose, maltose and sorbitol (Figure 3.2 a, b, c and d) and extremely low levels of manitol, melezitose, melibiose, raffinose and erlose were also found (Table 3.4).

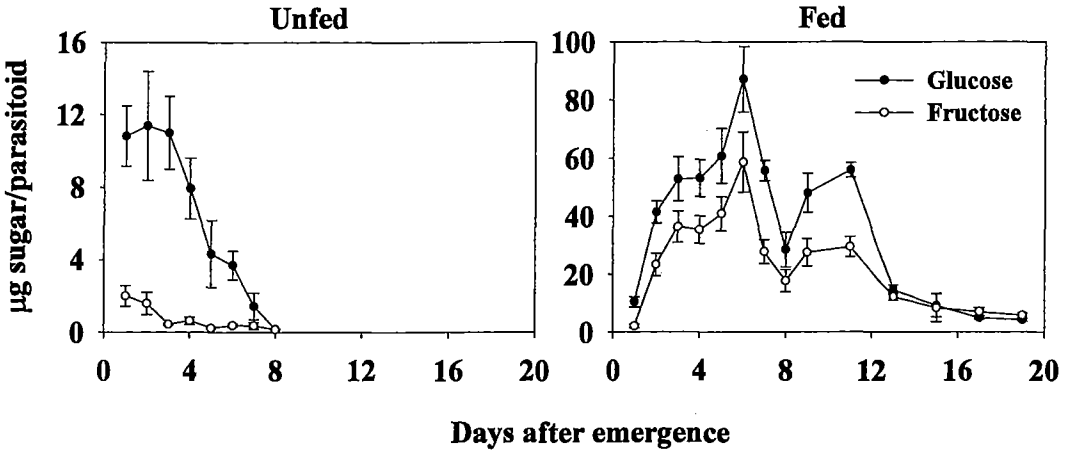

Figure 3.2: Glucose and fructose levels change in honey-fed and unfed *M. hyperodae.*

Table 3.4: Sugars detected in minor quantities in continuously-fed *M. hyperodae.*

Days	Mean sugar levels µg/parasitoid				
	Manitol	Melezitose	Melibiose	Raffinose	Erlose
1	0.00	0.00	0.00	0.00	0.00
2	0.02	0.16	0.00	0.25	0.25
3	0.00	0.19	0.00	0.15	0.15
4	0.16	0.15	0.02	0.13	0.13
5	0.00	0.16	0.00	0.32	0.32
6	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00
8	0.00	0.08	0.00	0.00	0.00
9	0.24	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00
15	0.00	0.03	0.00	0.02	0.02
17	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00

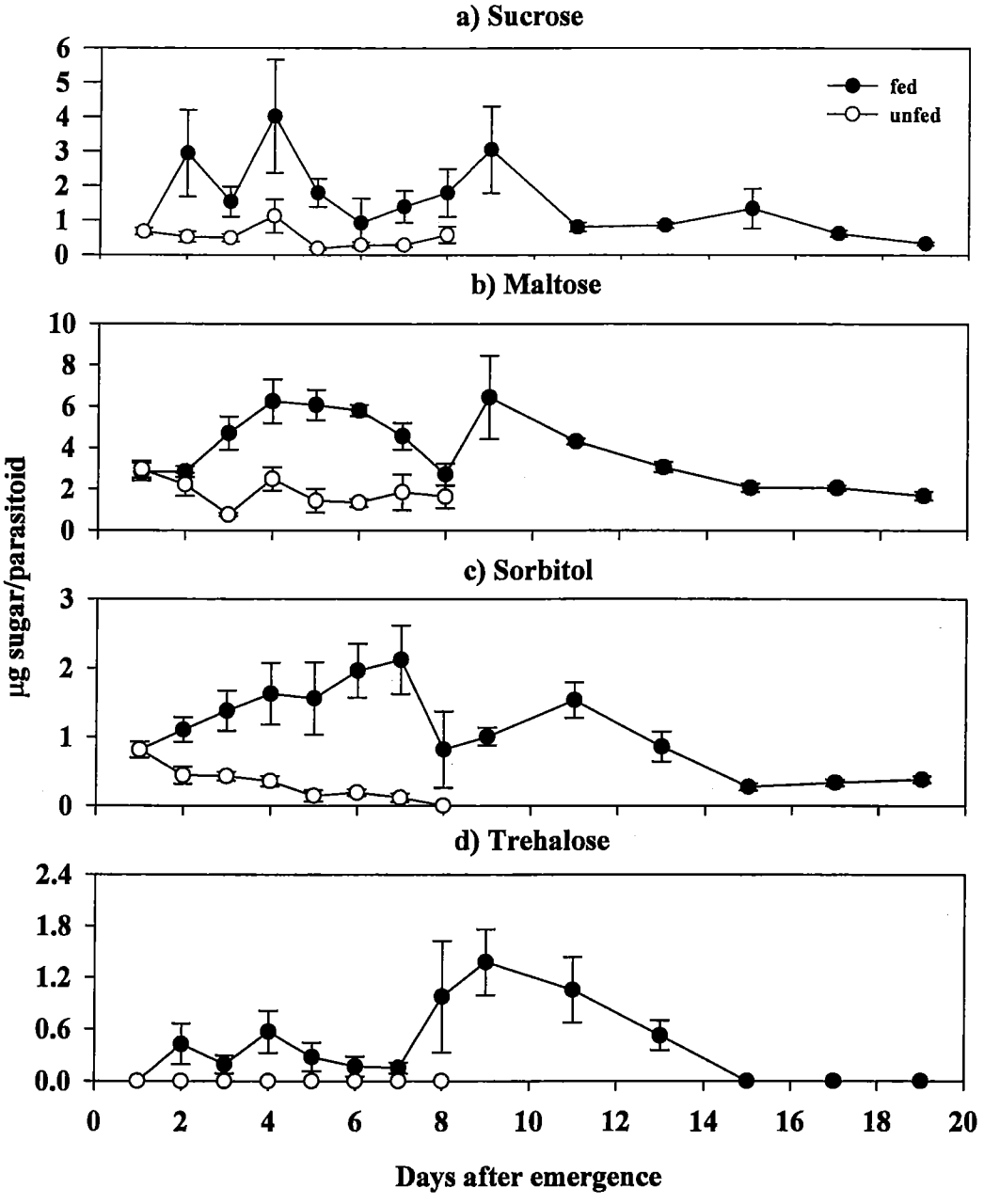

Figure 3.3: Changes in levels of sucrose, maltose, sorbitol and trehalose over the lifespan of fed and unfed parasitoids.

3.3.4 Sugar levels in parasitoids starved after having fed on buckwheat

Sugar analysis in parasitoids that had been starved for various intervals after having fed from buckwheat showed that their sucrose levels were 23.6 ± 1.86µg (n = 3) and 8.9 ± 0.61µg (n = 3) per parasitoid at 0 and 0.5h, respectively. This was a significantly higher ($P < 0.01$) than sucrose levels in parasitoids tested from 1 to 12h, which had low sucrose levels (Figure 3.4).

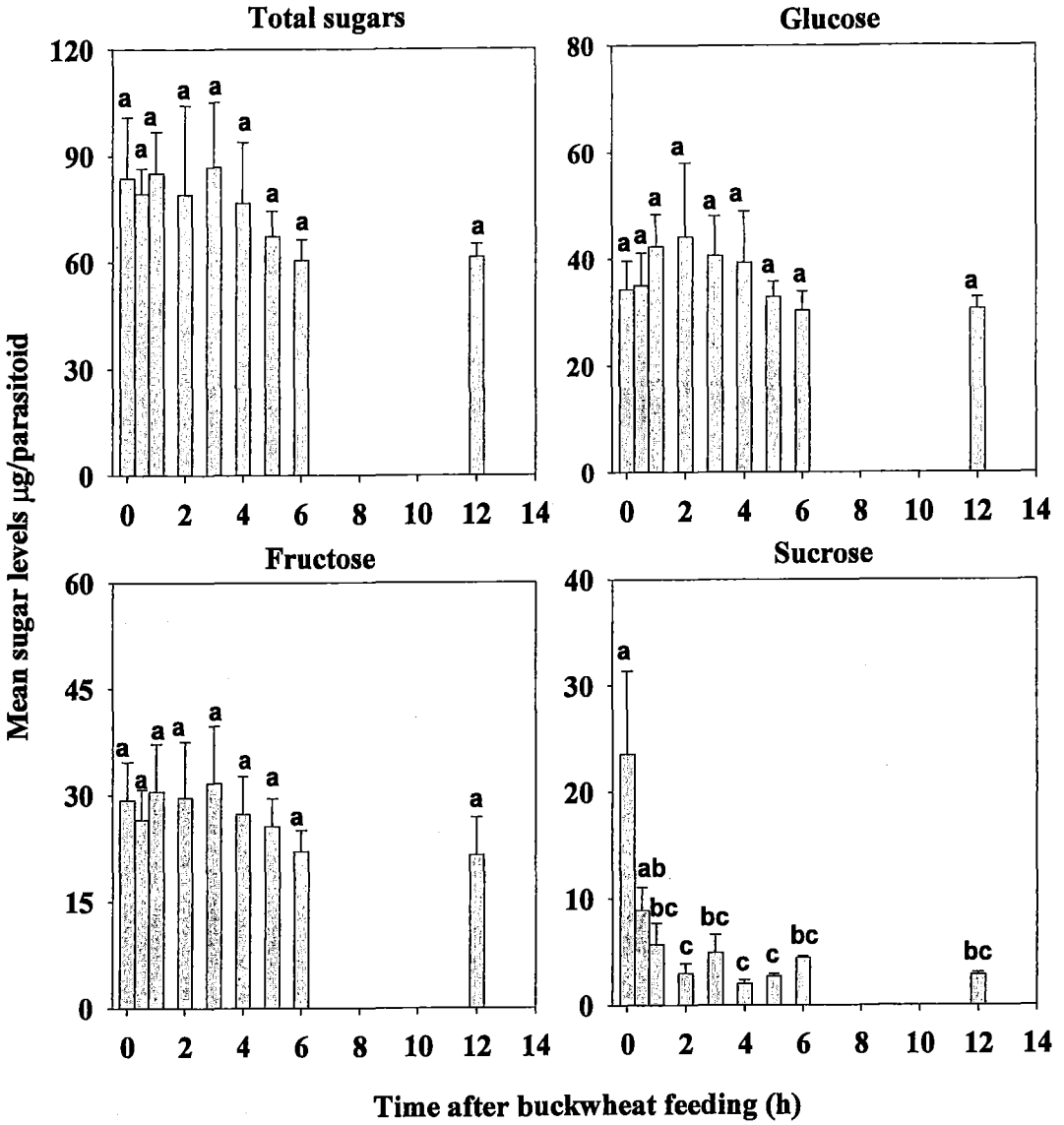

Figure 3.4: The mean 1) total sugars, 2) glucose, 3) fructose and 4) sucrose levels detected in *M. hyperodae* starved after buckwheat feeding. Bars sharing the same letters do not differ at $P = 0.05$. Error bars = +SE.

3.4 Discussion

3.4.1 Sugar levels in newly emerged parasitoids

Sugar levels in newly emerged *M. hyperodae* were dominated by glucose, which comprised 60% of the weight of all sugars. Fructose, maltose, and sucrose were present in considerably lower amounts. This sugar composition in newly emerged parasitoids could be due to the levels of

sugars acquired from the host during the larval stage, or as a result of metabolism occurring in *M. hyperodae* shortly after eclosion. In addition, the presence of the sugar alcohol sorbitol could be a remnant of the cold protection left over from the dormant stage of the *M. hyperodae* larva. Sorbitol is an important sugar alcohol that provides freezing resistance for insects (Chino 1957; Steele 1981).

3.4.2 Effects on body sugar levels in unfed parasitoids

In previous laboratory experiments, unfed *M. hyperodae* lived 10 days with water only (Hodgson *et al.* 1993; Vattala *et al.* 2004). However many other parasitoid species studied in similar experiments lived only 2-3 days (Siekmann *et al.* 2001; Scarratt 2002; Steppuhn & Wäckers 2004; Lavandero *et al.* in press). HPLC analysis showed that female *Cotesia glomerata* (L.) and *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) had almost depleted their body sugars by the second day following emergence (Steppuhn & Wäckers 2004). In contrast, body sugars in unfed *M. hyperodae* declined to 50% of their initial level in about five days, and to 15% by the eighth day. This apparently more gradual decline in total body sugar compared with *C. glomerata* and *M. mediator* (Steppuhn & Wäckers 2004) may facilitate the survival of *M. hyperodae* in the absence of sugar sources. This might be an adaptation related to the presumed natural habitat of this parasitoid and such habitats are often depauperate in suitable nectar sources, like many agricultural landscapes (van Emden 1990).

3.4.3 Effects on body sugar levels in continuously-fed parasitoids

Differences in fructose levels between fed and unfed parasitoids clearly distinguished the two groups. After 15 days fructose levels exceeded glucose levels and stayed high until the parasitoids died. In contrast, after 15 days the total sugar level in fed parasitoids did not differ from unfed individuals (Table 3.3).

3.4.4 Sugar levels in parasitoids starved after having fed on buckwheat

Newly emerged, unfed parasitoids always had lower fructose levels than fed individuals. The major difference observed in buckwheat-fed parasitoids were high sucrose levels compared with

honey-fed and unfed parasitoids. The parasitoids analysed immediately after having fed on buckwheat showed very high sucrose levels. This is consistent with the results in Chapter 2 that showed buckwheat nectar contained high sucrose. However, sucrose levels rapidly declined within 1h of feeding, then stabilised, and showed approximately similar levels after 1 to 12h (Figure 3.4). Therefore, it appears that the high sucrose observed in *M. hyperodae* just after feeding must have come from undigested sucrose in the gut. These sucrose levels probably declined after one hour due to hydrolysis and absorption of two hexose units (glucose and fructose) through gut walls. This suggests that the 'lifetime' of gut sugars in *M. hyperodae* is one hour or less.

3.4.5 Comparison of sugar level changes in fed and unfed parasitoids

Olson *et al.* (2000) and Casas *et al.* (2005) showed that at the time of death, unfed parasitoids had very low total sugar levels. Casas *et al.* (2005) emphasised that the most probable reason for the death of the unfed parasitoid is a low carbohydrate level. At the time of death, *M. hyperodae* also had low total sugar levels (2.5 $\pm$ 0.33µg). The present study further reveals that, in addition to low total sugar levels, unfed *M. hyperodae* had extremely low levels of glucose and fructose at death. Levels of these two monosaccharides had reached almost zero (glucose 0.2 $\pm$ 0.01µg and fructose 0.2 $\pm$ 0.01µg). The contribution to the total sugars from glucose and fructose was only 13% at death. This suggests they were unable to convert the remaining disaccharides (mainly sucrose at 0.57 $\pm$ 0.237µg and maltose at 1.63 $\pm$ 0.558µg) to useable monosaccharides (Figure 3.3).

In contrast, total sugar levels remained high (12.1 $\pm$ 1.484µg) at the time of death of fed parasitoids (Table 3.3 and Figure 3.3). Therefore, it appears that sugar is not the limiting resource which causes death. There could be many reasons for the death of fed parasitoids. For example, Olson *et al.* (2000) suggested that it is possible that lowering lipid levels in fed parasitoids could be the main cause of death. Giron & Casas (2003) endorsed this statement by concluding that "in all the parasitoids in which lipogenesis was studied, none was able to synthesis lipids from sugars". Although, declining lipid levels in fed parasitoids may indicate approaching death, they are not necessarily the cause of death (J. Casas pers. comm.).

3.4.6 Total sugar as a tool to discriminate fed and unfed parasitoids

In a previous study, unfed *C. glomerata* and those starved for three days after having being fed for two days had approximately equal total sugar levels. This shows that if parasitoids are starved for several days after feeding, total sugar levels can decline to those of the unfed ones (Steppuhn & Wäckers 2004). Comparison between fed and unfed *C. glomerata* was possible only for three days, since this was the longevity of unfed parasitoids. Although Steppuhn & Wackers (2004) showed that total sugars cannot be used to distinguish feeding history (whether parasitoids are fed at any stage) of unfed and starved (after feeding) parasitoids, they concluded that it is possible to distinguish the nutritional state (i.e., sugar quantity in the body which is an indication of a usable energy levels) of fed and unfed parasitoids. In contrast, HPLC analysis of *M. hyperodae* showed that total sugar levels cannot be used to distinguish, either feeding history or nutritional state, between fed and unfed parasitoids (Table 3.3). This is because, although fed and unfed *M. hyperodae* could be distinguished on the basis of total sugar levels for a few days, as was the case for *C. glomerata* (Steppuhn & Wackers 2004), older fed (parasitoid aged more than 15 days) and unfed (on days two, three and four) *M. hyperodae* had similar sugar levels (Table 3.3). The higher rate of glucose metabolism observed in fed *M. hyperodae* (Figure 3.2) from day 15 until death brought the total sugar levels below the levels of unfed parasitoids (Table 3.3). For the *C. glomerata* work (Steppuhn & Wackers 2004), data collection was discontinued by day 14, so the possibility of a later decline in sugar levels of fed parasitoids could not be investigated. Therefore, it is not certain that nutritional state is a reliable tool to discriminate fed *C. glomerata* from unfed ones at later ages.

3.5 Conclusions

Most work on insect physiology has been carried out on a few 'model' insects, especially in the families such as Lepidoptera, Coleoptera and Diptera and, in Hymenoptera, mainly honey bees. Not until recently has parasitoid physiology begun to be studied in detail (Wäckers & Swaan 1993; Wäckers 1998; Olson *et al.* 2000; Wäckers 2001; Scarratt 2002; Giron & Casas 2003; Wäckers & Steppuhn 2003; Winkler *et al.* 2003; Steppuhn & Wäckers 2004; Casas *et al.* 2005). These studies pioneered the elucidation of lifetime nutrient changes in adult parasitoids.

Understanding parasitoid nutritional requirements and the metabolic pathways of nutrients can help researchers in biological control to manipulate natural enemies and manage pest populations efficiently. However, the current level of information shows that very little is known about many physiological aspects of natural enemies. The so-called 'insect haemolymph sugar', trehalose, was not detected in fed or unfed *C. glomerata* or *M. mediator* (Steppuhn and Wackers 2004) nor in newly emerged and unfed *M. hyperodae* in this study. So, it is timely to study more natural enemies in this regard and if necessary, evaluate some of the commonly used terminology such as insect 'haemolymph sugar'.

As parasitoids age, their physiological state appears to change. Clarke & Maynard-Smith (1961) developed the idea of a "threshold theory" of ageing of insects. Their study with *Drosophila subobscura* Collin showed that the initial phase of ageing is irreversible and proceeds at a rate that is independent of the temperature at which they were reared (between 15°C and 30°C). This is possible only until the "vitality" of an individual reaches the "threshold" level below which it can no longer maintain a steady state. It could be possible that the "threshold theory" of ageing comes into play when sugar levels in parasitoids reach a certain point. Probably, it is at that stage that normal metabolic functions in the parasitoid body are malfunctioning. One clue, which was shown in the current study, is the sudden glucose level reduction in old parasitoids compared with fructose (Figure 3.2). Therefore, more research needs to be done to understand the link between ageing and nutrient level changes in parasitoids, fed individuals in particular. Such research should inform still further conservation biological control research by allowing for the exploration of parasitoid 'fitness' in time and space and in relation to the extent of flower feeding.

The gut sugar retention time in *M. hyperodae* (section 3.4.5) may imply that they might not be interested in feeding on sugars for up to one hour. Therefore it is useful to conduct a behavioural experiment to understand switching frequencies between feeding and host searching. It could be possible that higher sugar levels would help parasitoids to increase their searching efficiency in addition to longevity. Laboratory experiments were conducted in enclosed cages where parasitoids activities were restricted. A similar experiment could be conducted to evaluate the changes of body sugar levels of fed and unfed parasitoids in a semi-field environment. Such an experiment could provide a better understanding of parasitoid energy metabolism in field

conditions. Sugar-level in parasitoids starved after having fed from buckwheat were studied for up to 12 hours in this study. This aspect should be investigated further keeping starved parasitoids for several days.

CHAPTER 4

THE EFFECTS OF BUCKWHEAT ON THE ABUNDANCE OF *Microctonus hyperodae*

4.1 Introduction

It has been shown in many studies that encouraging flowering weeds adjacent to crops may increase the level of pest parasitism (van Emden 1962, 1965; Jervis *et al.* 1993). Irvin *et al.* (2000) recorded significantly high *Dolichogenidea tasmanica* (Cameron) on yellow sticky traps near buckwheat plots in an apple orchard. Although there were no significant differences between buckwheat and control plots, Berndt *et al.* (2002) found that male *D. tasmanica* were more abundant in buckwheat plots than in control plots. Parasitoids have been known to use visual (Wäckers 1994) and olfactory (Takasu & Lewis 1996) cues to locate non-host food sources, such as flower nectar. Therefore it is assumed that a greater number of parasitoids could be expected in the presence of flowers (van Emden 1962; Hirose 1966; Chaney 1998; Stephens *et al.* 1998).

In conservation biological control (CBC), it is expected that parasitoids would be attracted to, and benefit from, floral resources to improve the efficacy of biological control. Hence, the first step towards a successful CBC programme is to ensure natural enemies will aggregate at floral resources. Parasitoid aggregation near floral resources is the first step in a logical hierarchy for measuring the success of CBC (Wratten *et al.* 2003a; Wratten *et al.* 2003b). This hierarchical order has already presented in Section 1.6. This chapter focuses on evaluating the extent to which parasitoids aggregate in the vicinity of buckwheat plots in the field.

Top-down effects (the 'enemies hypothesis' of Root (1973)) can result from high parasitoid aggregation via two possible mechanisms: First, resource subsidies could attract parasitoids, which then aggregate around these resources (Liang & Huang 2000) and, therefore, increase their local density. High numbers of parasitoids could result in higher parasitism rates (Tylianakis *et al.* 2004). Second, parasitoid 'fitness' could be enhanced by feeding from resource subsidies

(Kean *et al.* 2003; Tylianakis *et al.* 2004; Gurr *et al.* 2005). This could result in high numbers of attacks per parasitoid during its lifetime, through increases in adult longevity and fecundity (Tylianakis *et al.* 2004).

Spatial and temporal patterns of relative abundance of insects have been studied using a wide range of methods, including sticky traps (Samways 1986; Robin & Mitchell 1987; Rice & Michailides 1988; Heng Moss *et al.* 1999). The adhesive surface of the trap captures flying and wind-blown insects (Southwood 1978). The trap colour plays an important role in attracting insects (Kirk 1984). For example, wood borers and biting flies prefer dark colours, such as black and red, while insects associated with plant foliage generally prefer yellow. Insects associated with flowers, however, show variability in their response to traps and there is evidence that they are more attracted to colours such as white, yellow and blue (Kirk 1984). Nonetheless, yellow sticky traps have successfully been used to study parasitoid abundance in many situations (Trimble & Brach 1985; Samways 1986; Irvin 1999; Berndt *et al.* 2002).

Many laboratory studies have shown that flowering plants can enhance one or more measures of parasitoid 'fitness'. For example, longevity has been increased in almost all studies and fecundity has been increased in some of them (see Table 2.1 for details). The ultimate challenge faced by biological control workers is to make this happen in the field. There were few examples that the provision of floral resources demonstrated a reduction in pest abundance (White *et al.* 1995; Wysse 1995; Hickman & Wratten 1996; Chaney 1998; Patt *et al.* 1999). However, some studies showed no effect (Pimbert & Srivastava 1989), or occasionally increased pest abundance with flowers (Baggen *et al.* 1999). The objective of this chapter, however, is to measure the parasitoid abundance in the vicinity of buckwheat plots.

4.2 Materials and methods

4.2.1 Site description

Two field experiments were conducted simultaneously from mid September 2003 to April 2004. Three 40 x 40m (approximate size) paddocks of perennial ryegrass (*L. perenne*) were marked out at the AgResearch Farm (Field Site 1) in Boundary Road, Lincoln, Canterbury, New Zealand

(Figure 4.1). A private farmer was contacted through Wrightsons Research for the second field site. This farm was situated in Robinsons Road (Field Site 2), Ladbrooks, Canterbury, New Zealand (Figure 4.2). Field Site 2 was a large single block with *L. perenne*. Low-endophyte ryegrass had been sown at both sites more than two years previously and this usually supports high *L. bonariensis* populations. Initial *L. bonariensis* sampling at both field sites revealed nearly 30% parasitism rates.

4.2.2 Experimental design

Two different experimental layouts were used in these farms (Figures 4.1 and 4.2) because the land allocated for the field experiments in these farms varied in size. Plots in which buckwheat was to be sown were demarcated at both field sites and herbicide was sprayed two weeks before the intended sowing date to clear all grass and weeds in those areas. All plots were cultivated, and buckwheat was sown on 17 and 19 September 2003 at Field Sites 2 and 1, respectively. Sowing dates were selected based on the data of Bowie *et al.* (1995) so that the buckwheat would flower by mid November to synchronise with the emergence of adults from the over-wintered generation of *M. hyperodae* (see Figure 1.2, Chapter 1). Two additional sowings were made in November and January to maintain the availability of flowers by producing three cohorts of buckwheat plants throughout the field season. Watering was done when necessary to maintain healthy plants throughout the sampling period. All weeds and other flowering plants were manually removed from the buckwheat plots and experimental paddocks. To avoid any damage to buckwheat plants from livestock, steel gates were used to fence them at Field Site 1 (Plate 4.1). An existing electric fence was extended around the buckwheat plots at Field Site 2 (Plate 4.2) for the same purpose.

Double-sided yellow sticky traps ('Trappit', Agrisense-BCS Ltd., Treforest Industrial Estate, Pontypridd, Mid-Glamorgan, U.K., supplied by Fruitfed Supplies Ltd., New Zealand) measuring 200 mm x 245 mm were used to capture parasitoids. Traps were set up at both farms for seven days prior to each collection date (Plate 4.2 and Plate 4.3). The placement of the sticky traps on the ground was determined by preliminary experiment conducted to capture parasitoids at three different heights (0, 10 and 20cm) above ground level.

Field Site 1 – AgResearch Farm

The three experimental paddocks at Field Site 1 were large enough to drill an 8.3 x 8.3m buckwheat plot in the centre of each paddock (Figure 4.1). Four transects radiating from each side of each these plots were marked with bamboo stakes at 0, 1, 2, 5, 10 and 15m. Sticky traps placed vertically at ground level with one side facing the buckwheat plot and the other facing in the opposite direction.(Figure 4.1, Plate 4.4). Each transect had six traps and each paddock had four transects.

Field Site 2 – Robinsons Road Farm

At Field Site 2, three 10 x 2m buckwheat plots were drilled, leaving an 80m space between each. There were two transects for each buckwheat plot and those were placed one opposite to other. Transects radiated at 90° from each of the two long sides of each of the three buckwheat plots, with bamboo stakes on each transect marking 0, 1, 2, 5, 10, 15 and 20m from the edge of the plot. It was possible to use an additional trap at Site 2 compared to Site 1 because of the larger paddock. Therefore one transect had seven traps (Figure 4.2) .

Sticky traps were collected for analysis from both sites on four days, two in November and two in December 2003 at both farms. The traps were numbered and placed in a refrigerator at 4°C pending analysis. Traps were carefully examined later under a microscope at 50X magnification in the laboratory to identify and count the *M. hyperodae*.

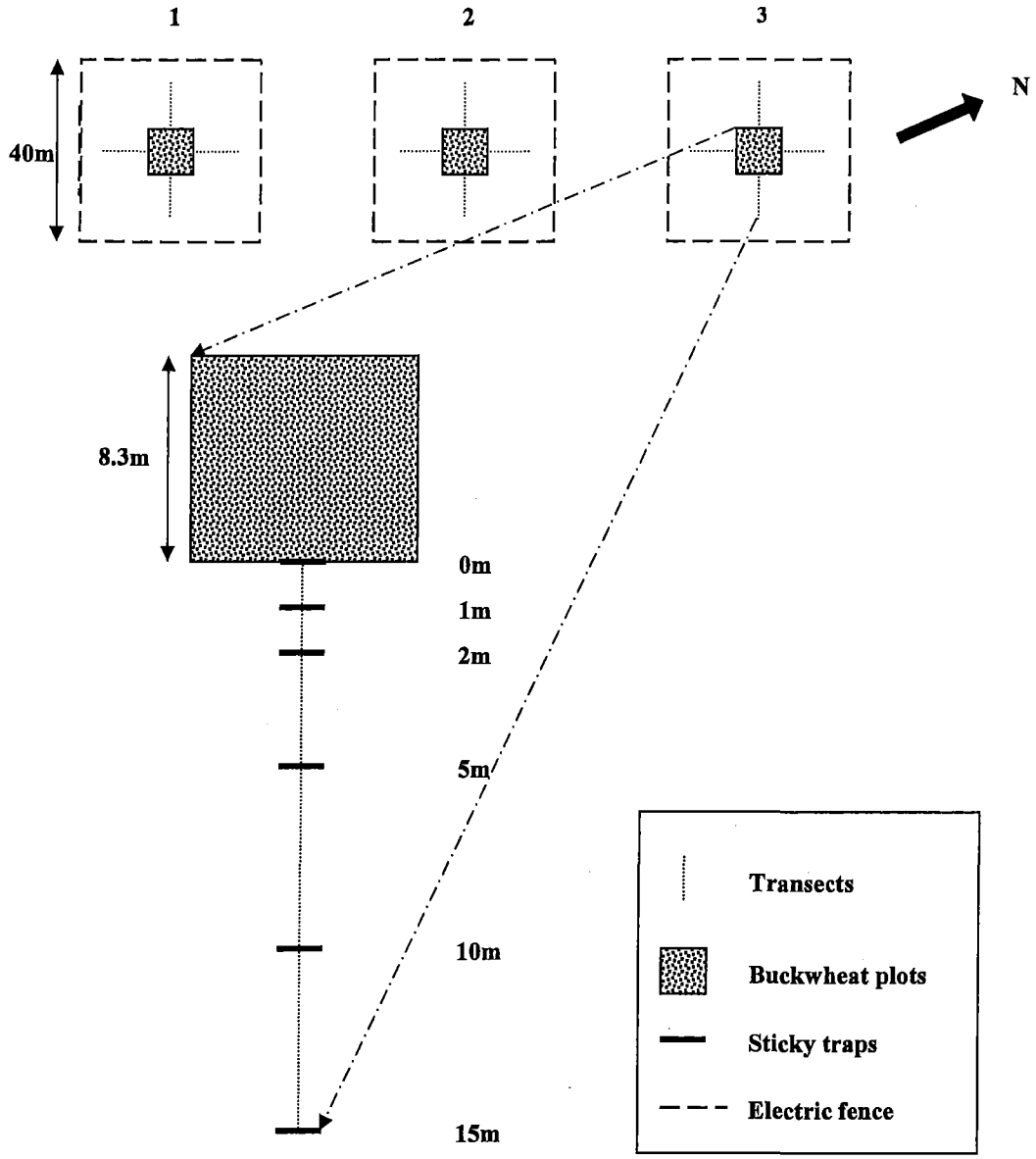

Figure 4.1: The experimental layout used at Field Site 1 (AgResearch Farm). Three separate paddocks (1, 2 and 3) were used, and the size of each paddock was approximately 40 x 40m. The 8.3 x 8.3m buckwheat plot was in the centre of each paddock. Four transects radiated from each side of each of the plots' buckwheat-grass margins. All three paddocks were surrounded either by adjacent paddocks or by grass races.

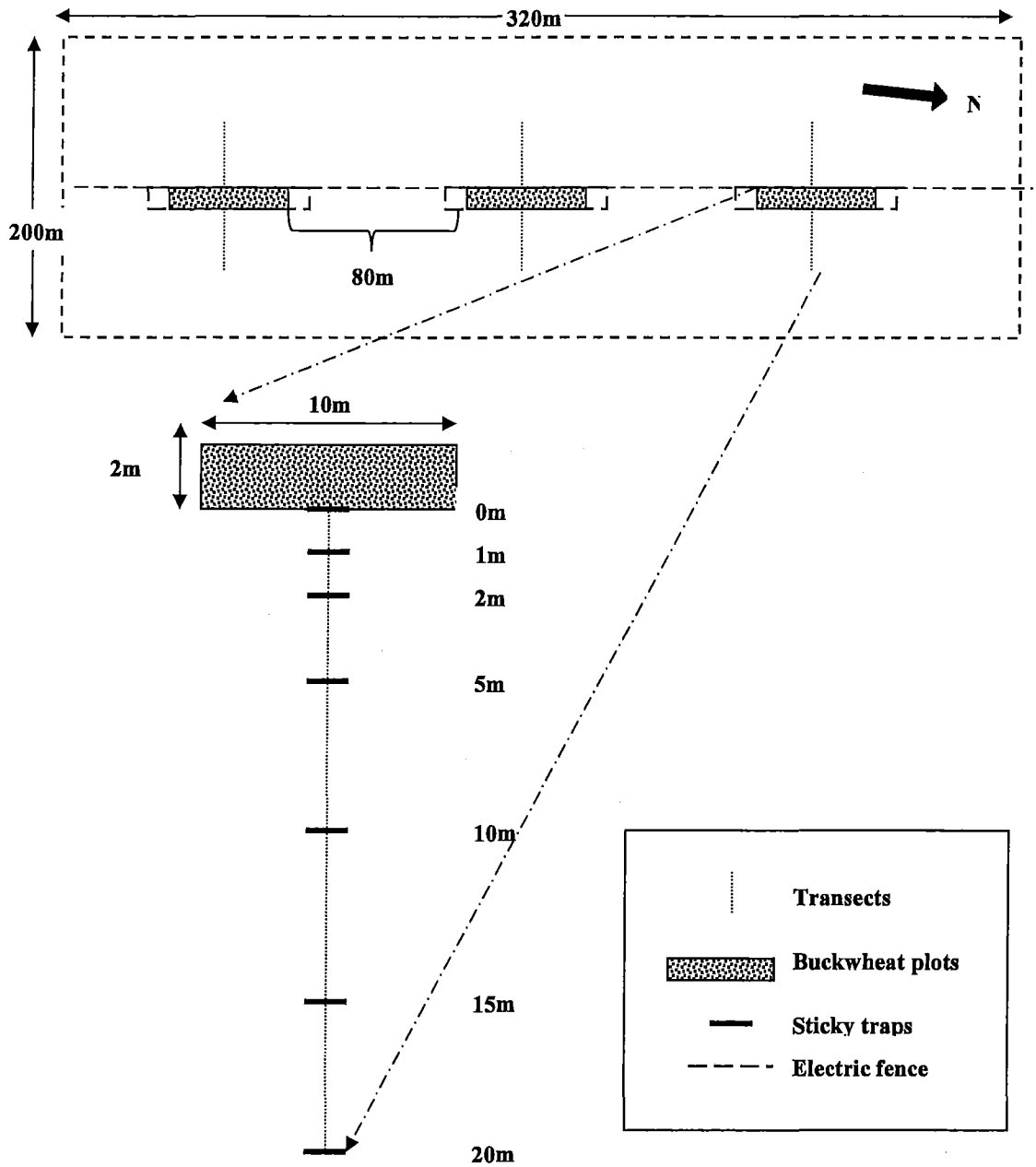

Figure 4.2: The experimental layout used at Field Site 2 (Robinsons Road). The size of each buckwheat plot was 10 x 2m. The buckwheat plots were sown adjacent to an electric fence which the paddock (approximately 320 x 200m). Two transects were on opposite sides of the plots. The experimental block was surrounded by grass paddocks.

4.2.3 Statistical analysis

The appropriateness of parametric analysis was confirmed by inspection of the normality of the residual plots. A generalised linear model (GLM) for poisson distribution with a log link was

used to determine whether there were any effects from transects, collection date and distance from buckwheat, on the number of parasitoids captured. Based on the GLM results, data for all transects in each field site were pooled. To describe the change in parasitoid numbers with increasing distance from the buckwheat plots a double exponential curve was fitted using GenStat V.7.

Justification for the type of curve

Parasitoid numbers decreased with increasing distance from the buckwheat plots at both experimental sites. However, beyond a certain distance parasitoid numbers were increased. Since this effect occurred at both sites and the minimum number of parasitoids obtained from fitted values occurred at approximately similar distances from buckwheat plots the phenomenon was considered to be a real effect rather than due to sampling. For this reason it was decided to fit a curve that would take account of both the initial rapid decline in parasitoid numbers with increasing distance, the gradual levelling off in numbers followed by the observed increase in numbers. Various curves were tried and the double exponential was chosen, this curve taking account of all aspects of the parasitoid response. Fitting this curve then enabled estimation of the distance at which the number of parasitoids was a minimum. It was then possible to examine the spatial effects of buckwheat on parasitoids.

4.3 Results

At Field Site 1, 72 traps were collected on each date, totalling 288 traps over four days. The total number of parasitoids captured was 148 (0.06 parasitoids per day per trap). At Field Site 2, the total number of traps used was 168 (comprising 42 traps per collection date). The total number of parasitoids captured from these traps was 93 (0.08 per day per trap). GLM showed that there was no significant effect of either collection date or transect on the number of parasitoids captured ($P > 0.05$) However, there was a significant effect of distance from buckwheat on number of parasitoids captured at different distances from buckwheat plots ($P < 0.01$). The mean number of parasitoids captured at different distances, and fitted values obtained from a double exponential curve [$Y = a + b_1 \exp(-cx) + b_2 \exp(dx)$] are shown in Tables 4.1 and 4.2.

Table 4.1: The mean number of parasitoids captured at Field Site 1 at varying distances from buckwheat plots and the fitted values obtained from the curve.

Distance from flowers (m)	Mean number of parasitoids	± SE	Fitted values
0	1.42	0.07	1.42
1	0.71	0.07	0.71
2	0.35	0.07	0.36
5	0.06	0.04	0.06
10	0.04	0.03	0.04
15	0.50	0.07	0.50

Capture rates were very low at 5 and 10m at Field Site 1. The fitted values hardly differed from the means.

Table 4.2: The mean number of parasitoids captured at Field Site 2 at varying distances from buckwheat plots and the fitted values obtained from the curve.

Distance from flowers (m)	Mean number of parasitoids	± SE	Fitted values
0	1.46	0.12	1.47
1	0.75	0.11	0.69
2	0.33	0.10	0.42
5	0.29	0.09	0.27
10	0.29	0.09	0.27
15	0.29	0.09	0.30
20	0.46	0.10	0.46

Capture rates were low at 5, 10 and 15m at Field Site 2. However at 5 and 10m, these values were not as low as were those for Site 1. Although some fitted values were different from the mean number of parasitoids captured, they were similar at 0 and 20 m (Table 4.2). The lowest fitted values were observed at 5 and 10m.

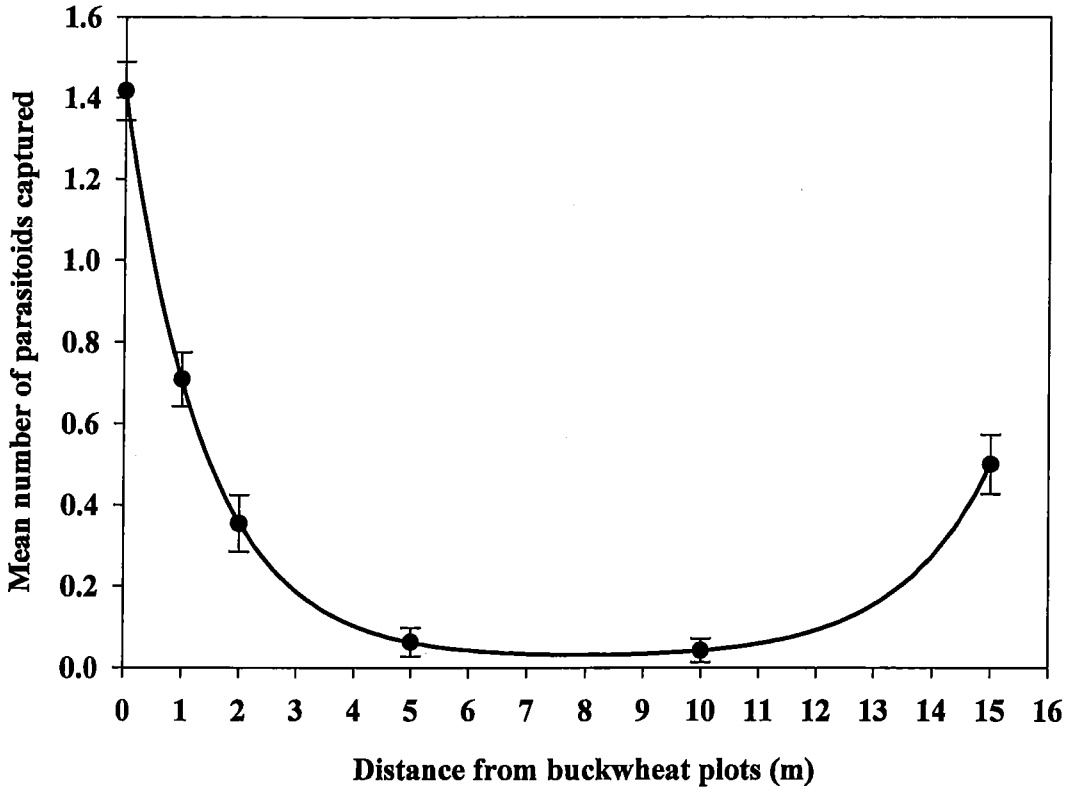

Figure 4.3: The curve shows the fitted values of *M. hyperodae* at Field Site 1 at different distances from buckwheat plots (r^2 = 0.56) and follows a double exponential path and reaches its minimum at 8 m, estimated as the value of x where dy/dx = 0. Points with error bars (± SE) show actual mean values.

The mean number of parasitoids captured at the margins of the buckwheat plots was 1.42 per trap. The number of parasitoids declined at intermediate distances from the buckwheat plots before increasing again in the traps furthest from the plots. The minimum number of parasitoids was captured at 10m and was 0.04 per trap. However, the fitted curve suggested that the minimum number of parasitoids was present at 8m away from the flower plots. Thereafter the number increased again and reached 0.50 parasitoids per trap at 15m (Figure 4.3).

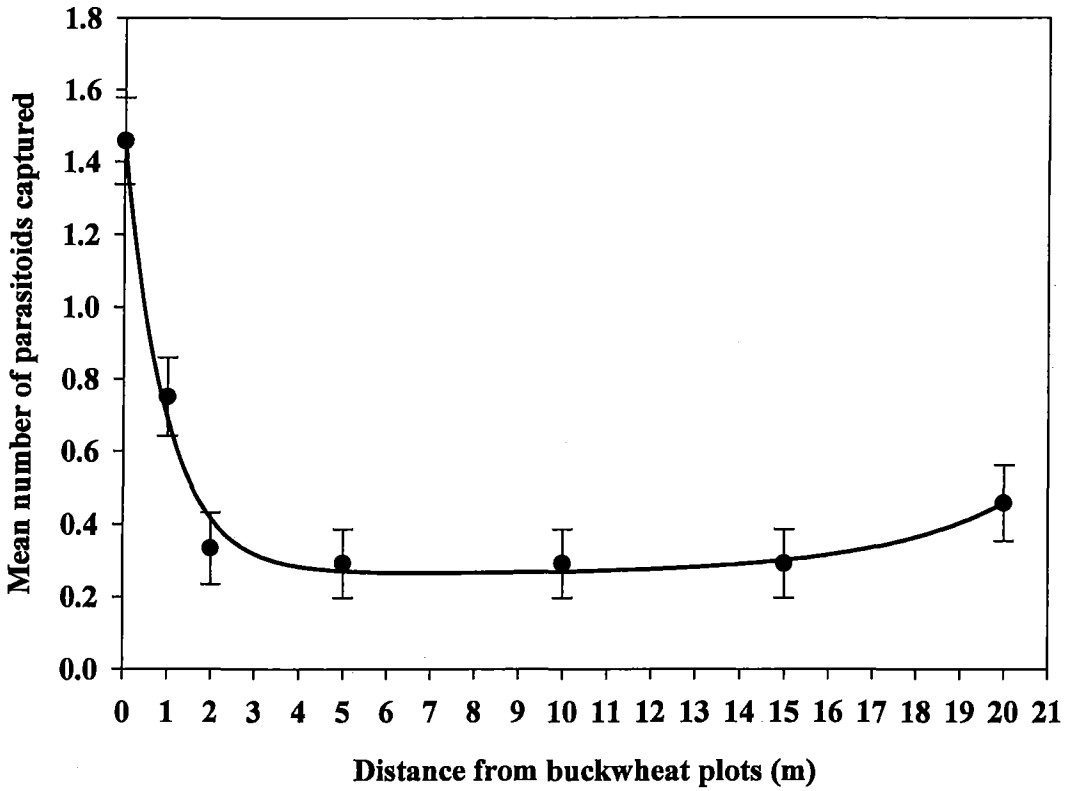

Figure 4.4: The fitted values of *M. hyperodae* capture at Field Site 2 at different distances from buckwheat plots ($r^2 = 0.42$). The curve follows a double exponential path and reaches its minimum at 7m, estimated as the value of x where dy/dx = 0. Points with error bars (± SE) show actual mean values.

The mean number of parasitoids captured at the margins of the buckwheat plots was 1.46 per trap. The number of parasitoids captured at different distances from buckwheat plots followed a similar pattern to Field Site 1. The mean number of parasitoids captured was similar at 5, 10 and 15m. However, the fitted curve suggested that the minimum number of parasitoids was present at 7m from the flower plot. Thereafter, the mean number increased again and reached 0.30 and 0.46 parasitoids per trap at 15 and 20m, respectively (Figure 4.4).

4.4 Discussion

The highest *M. hyperodae* abundance occurred at the transect point near the buckwheat plots suggesting that parasitoids are attracted to buckwheat plants. The mean number of parasitoids was significantly lower at 8m and 7m at Field Sites 1 and 2, respectively, compared with the nearest transect point to the buckwheat plots. There could be two explanations for this: First, the high aggregation of *M. hyperodae* closer to buckwheat plots could have occurred due to the influence of buckwheat through its olfactory or visual cues. Parasitoids have been known to use visual (Wäckers 1994) and olfactory (Takasu & Lewis 1996) cues to locate non-host food sources, such as flower nectar. Second, *M. hyperodae* which emerged closer to buckwheat plots lived longer as a result of nectar feeding and did not move more than 7 or 8m away from the plots after feeding. This latter possibility could be assessed by investigating the nutritional state and feeding history of parasitoids collected at different distances from buckwheat plots (see Chapter 3 and 5 for more details).

At both field sites, the mean number of parasitoids captured from the sticky traps in the middle of the transects (traps located at 5 and 10m) were lower than those caught from either end of the transects. The number was remarkably lower in Field Site 1 compared with Field Site 2 (Tables 4.1 and 4.2). These differences could have occurred due to size differences between the buckwheat plots in these two field sites. The size of the buckwheat plot in Field Site 1 was $69m^2$ (8.3 x 8.3m) and $20m^2$ (2 x 10m) in Field Site 2. Visual and olfactory cues may also have been greater with the larger buckwheat plots compared with the smaller ones. Therefore, it is possible that a greater number of parasitoids were attracted to buckwheat plots in Field Site 1. However, it appears that there was no effect from the size of the buckwheat plot beyond the 'influential zone' (7 to 8m which was derived from the fitted values, Figures 4.3 and 4.4). Therefore, parasitoid attraction to buckwheat seems to be a localised effect within the 'influential zone', however, and the effect appeared to be greater from the larger buckwheat plots.

At both sites the traps furthest from the buckwheat plots had higher numbers of parasitoids compared with those in the middle. There could be two reasons for this: First, buckwheat influenced *M. hyperodae* only up to 7 to 8m away. Therefore, all parasitoids within this distance may have been attracted to buckwheat, while parasitoids trapped beyond this point of influence probably reflected the natural abundance of *M. hyperodae* in the field. Second, some parasitoids

fed from flowers and dispersed and were subsequently attracted to yellow sticky traps after some period searching for food sources. Many studies showed that learning plays an important role, as parasitoids can use different visual and olfactory cues in relation to their physiological state and past experience (Lewis & Takasu 1990; Wäckers & Lewis 1994; Takasu & Lewis 1996; Sato & Takasu 2000; Tretuliano *et al.* 2004). The physiological state of parasitoids changes as they feed from floral resources. Wäckers (1994) stated that an insect's behavioural response towards food preference is determined by its innate preferences and physiological state. The innate response of parasitoids, however, could be changed depending on their experience due to rewards they obtained from prior feeding events (Takasu and Lewis 1993). Therefore, *M. hyperodae* may not have been attracted to some traps which they passed by due to their satisfactory physiological state. Parasitoids may resume flower seeking when their gut sugar levels reach a particular low threshold level. This second hypothesis can be tested by analysing the nutritional state and feeding history of parasitoids collected at varying distances from buckwheat plots.

4.5 Conclusions

This study shows that *M. hyperodae* aggregates at buckwheat plots in the field. However, the mechanism underling this aggregation is unknown. It could be possible that visual and olfactory cues are involved. Further research should be carried out to understand parasitoids' learning behaviour in the laboratory and in a semi-field environment with pasture and flowering plants. Other behavioural studies could be designed to record their feeding and host searching frequencies, which may help to understand how *M. hyperodae* allocates its time between these two activities.

It is not possible to confirm that parasitoids are actually feeding from buckwheat in the field, on the basis of the data obtained from this experiment. This can be done by collecting parasitoids at different distances from flowering plants and analysing them for their sugar levels. This will be the key question addressed in Chapter 5.

Three weeks after buckwheat sowing

Flowering buckwheat

Buckwheat plot with grazing sheep

Plate 4.1: The fenced buckwheat plots at Field Site 1 (experimental paddocks used at the AgResearch Farm during 2003 - 2004 field season).

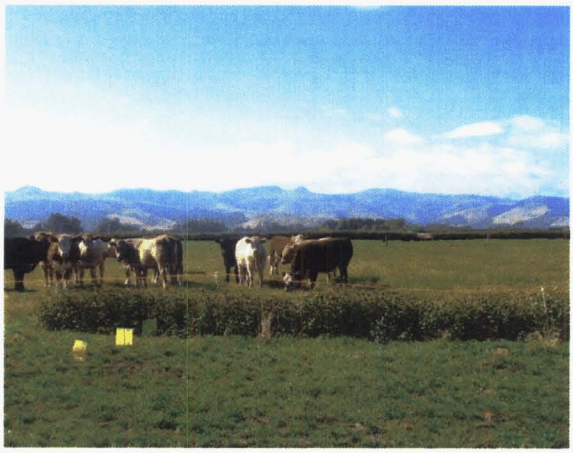

Plate 4.2: Flowering buckwheat plot showing extended electric fence at Field Site 2 during 2003 - 2004 field season (a private Farm at Robinsons Road, Ladbrooks).

Field Site 1

Field Site 2

Plate 4.3: Sticky traps set up at two field sites. Field Site 1 (above) and Field Site 2 (below).

Plate 4.4: Sticky trap showing captured insects in the field just before trap collection.

CHAPTER 5

ASSESSING THE FITNESS OF *Microctunus hyperodae* IN THE FIELD USING SUGAR ANALYSIS

5.1 Introduction

Parasitoids play an important role in reducing pest populations in agriculture and horticulture (Gurr & Wratten 2000). They occupy the third tropic level in food webs and, therefore, obtain energy reserves from the host, always as a parasitoid larva, and sometimes also as an adult via host-feeding. However, a considerable number of parasitoids do not host-feed, but depend on non-host foods (Jervis *et al.* 1993) such as plant provided 'resource subsidies' (Kean *et al.* 2003; Tylianakis *et al.* 2004; Gurr *et al.* 2005) for their energy demands as adults. There is substantial evidence that these resource subsidies, such as pollen and nectar are useful in enhancing one or more measures of parasitoid 'fitness' in the laboratory and field (Table 2.1, Chapter 2) and, therefore, have potential to enhance biological control.

Among these resource subsidies, floral and extrafloral nectar as well as honeydew are important energy sources. All are comprised primarily of sugar solutions, but they may differ in their qualitative and quantitative sugar composition. The disaccharide sucrose and its hexose units, glucose and fructose are the main components of floral and extrafloral nectar (Percival 1961). Also, some oligosaccharides (including melezitose, erlose, raffinose and trehalose) are present in honeydew, but are rarely found in nectar sources (Heimpel & Jervis 2005).

For many decades, biological control workers have recognised the importance of non-host food for increasing the efficacy of parasitoids in agricultural landscapes (Wolcott 1942; Hocking 1966). Many such areas are monocultures which are limited in resource subsidies. Ecosystem diversification in and around agricultural land could increase floral diversity and nectar availability. It is widely assumed that general ecosystem diversification can enhance the 'fitness' of parasitoids and increase parasitism rates, and some evidence supports this. Some field studies have shown that parasitoids visit flowers and feed on them (Jervis *et al.*

1993; Freeman Long *et al.* 1998), and others have shown that parasitism rates may increase as a result of intercropping or mixed cropping (reviewed by Powell 1986).

However, none of these studies have shown a direct link between parasitoid feeding on nectar sources and increased fitness in the field. Previous work showed that the availability of floral resources in the field resulted in increased parasitism rates (Leius 1967). It is assumed that the higher parasitism rates may have occurred due to the increased efficacy of parasitoids after feeding from those resources. Steppuhn and Wäckers (2004) provided a potentially powerful tool to investigate carbohydrate reserves in field-collected parasitoids. This tool is capable of providing information on whether the parasitoids had access to sugar meals during their lifetime (feeding history) and quantifying the parasitoids' total sugar levels (nutritional state). They investigated parasitoids from field-collected pupae and those reared in field cages. In both cases, fed parasitoids had a balanced glucose-fructose ratio (i.e., approximately 50:50), while glucose dominated the sugar levels in unfed individuals. *M. hyperodae* sugar analysis showed similar patterns (Chapter 3).

The main aim of this chapter is to analyse laboratory-reared parasitoids' sugar with emphasis on the sugar ratios used by Steppuhn and Wäckers (2004). This analysis will then be used to develop an analytical tool to discriminate between fed and unfed parasitoids. The ultimate objective of this chapter is to use this tool to measure *M. hyperodae* 'fitness' in relation to floral resources in the field.

5.2 Materials and methods

The first part of this chapter consists of developing possible tools to discriminate between fed and unfed parasitoids. The data obtained from the sugar analysis described in Chapter 3 were used to calculate different sugar ratios. Steppuhn and Wäckers (2004) examined glucose/fructose (g/f) and glucose/(glucose+fructose) (g/(g+f)) ratios in fed and unfed parasitoids and successfully used the latter to distinguish between them. Two ratios including the g/(g+f) ratio are derived for *M. hyperodae* in this chapter. In the current work, the methods of data collection for sugar ratio analysis are similar to those in Chapter 3 (Sections 3.2.1, 3.2.2 and 3.2.3). The second part of this chapter attempts to use these ratios and total sugar levels to understand the feeding history and nutritional state of field collected parasitoids.

5.2.1 Experimental design for parasitoid collection near buckwheat plots

Figure 5.1 shows the experimental layout used to collect parasitoids at the AgResearch farm (Field Site 1), which was one of the two sites used for the experiments described in Chapter 4. Buckwheat was grown in the middle of three ryegrass paddocks, as explained in Chapter 4. Four transects were marked, each radiating from one side of each buckwheat plot. The transect width was equal to the width of the buckwheat plots (8.3m) and its length was 14m. Overall, twelve transects were established, four in each paddock. The total area of each of these transects was 8.3 x 14m, and each was divided into seven areas, as shown in Figure 5.1, for insect collections. Therefore, the size of each collection area within each transect was 16.6m^2 (8.3 x 2m).

A suction sampler (Blower vac, SHERD N Vac Plus) (Plate 5.1) was used to vacuum parasitoids from each collection area following Phillips *et al.* (1998). A mesh bag (Plate 5.2) was placed in the suction tube to trap leaf litter and insects from which *M. hyperodae* was subsequently sorted.

Parasitoid collections were made on transects 1, 2 and 3, as shown in Figure 5.1 on the first collection date. Subsequent collections were made on numbers 4, 5 and 6 on the second collection date, 7, 8 and 9 on the third and 10, 11 and 12 on the fourth during November and December 2003, while avoiding the dates that sticky traps were in place (Chapter 4). The same routine was continued on two subsequent dates in January and February 2004.

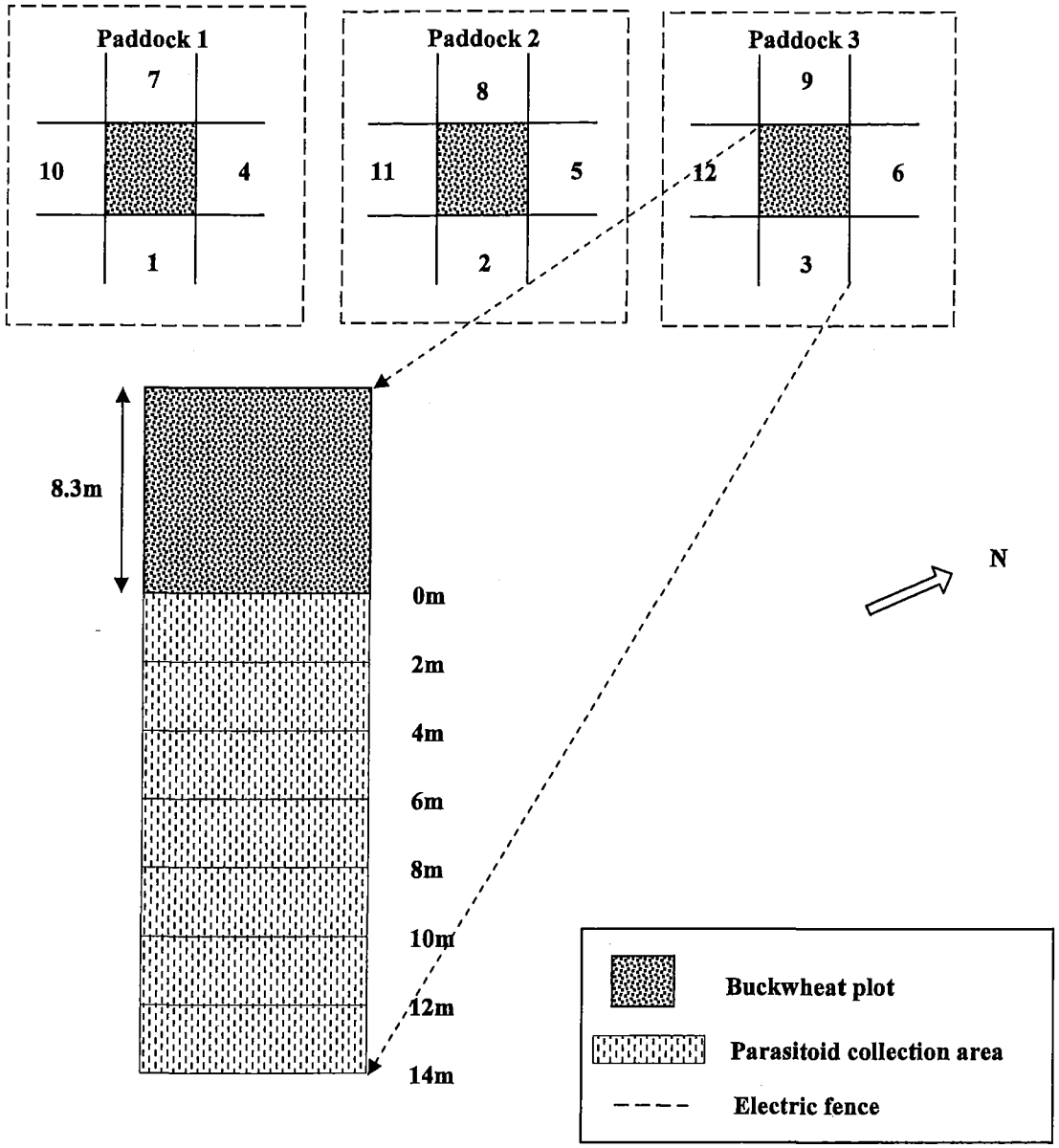

Figure 5.1: Experimental layout at the AgResearch farm. Size of each paddock (paddocks 1, 2 and 3) was approximately 40 x 40m. Parasitoids were collected from rectangular areas radiating from the buckwheat plot.

On each collection date, 21 bags were collected from three transects (seven bags from each). All bags were briefly checked for *M. hyperodae* in the field immediately after collection, and then checked again thoroughly in the laboratory within four to five hours of collection. Parasitoids were transferred to 5ml plastic vials, one parasitoid per vial. Each vial was given a number which corresponded to the distance from the buckwheat plot at which it was collected. While in the field, vials containing field-sorted parasitoids were stored in an insulated ice box until they could be transferred to a -80°C freezer. Parasitoids which were

sorted in the laboratory were immediately frozen at -80°C for subsequent HPLC analysis (see Chapter 3 for details of HPLC analysis).

5.2.2 Parasitoid collection from road-side verges

Parasitoids were collected by vacuuming grasses from road-side verges near Lincoln using the previously described suction device. Four collections were made, one in each month from mid November to mid February. All parasitoids were frozen at -80° C pending HPLC analysis (see Chapter 3 for details of HPLC analysis).

5.2.3 Statistical analysis

GenStat Version 8 was used for sugar analysis. The data showed lack of normality so non-parametric statistics were used. The sugar concentrations for parasitoids from different treatments and ages were compared pair-wise using a Mann-Whitney U-test. When testing for differences between ages, Kruskal-Wallis ANOVA was used.

5.3 Results

5.3.1 Glucose/(glucose+fructose) ratio in laboratory-reared parasitoids

The g/(g+f) ratio was 0.8 ± 0.02 (± SE) in newly emerged parasitoids, but it declined to 0.6 ± 0.03 on day two in unfed parasitoids, and to 0.4 ± 0.02 by day 19 in fed parasitoids. The ratio remained between 0.8 and 0.9 in unfed parasitoids from day two to six, then declined to 0.7 and 0.5 on days seven and eight, respectively (Figure 5.2).

The (g/(g+f)) ratio in fed and unfed parasitoids significantly differed from day two (24h after feeding) and day six ($P < 0.01$), but not on days seven or eight ($P > 0.05$) (Figure 5.3).

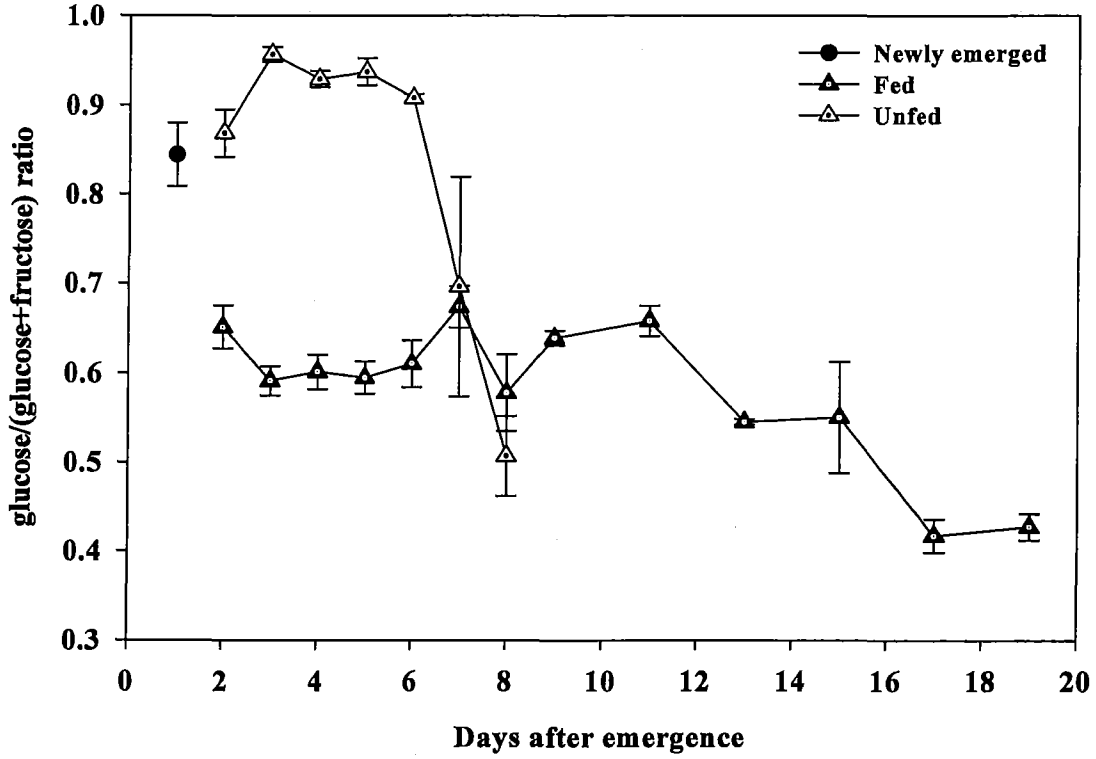

Figure 5.2: The glucose/(glucose+fructose) ratio in newly emerged, fed and unfed parasitoids. On days seven and eight, the g/f ratios of fed and unfed parasitoids were not distinguishable. Error bars = ±SE.

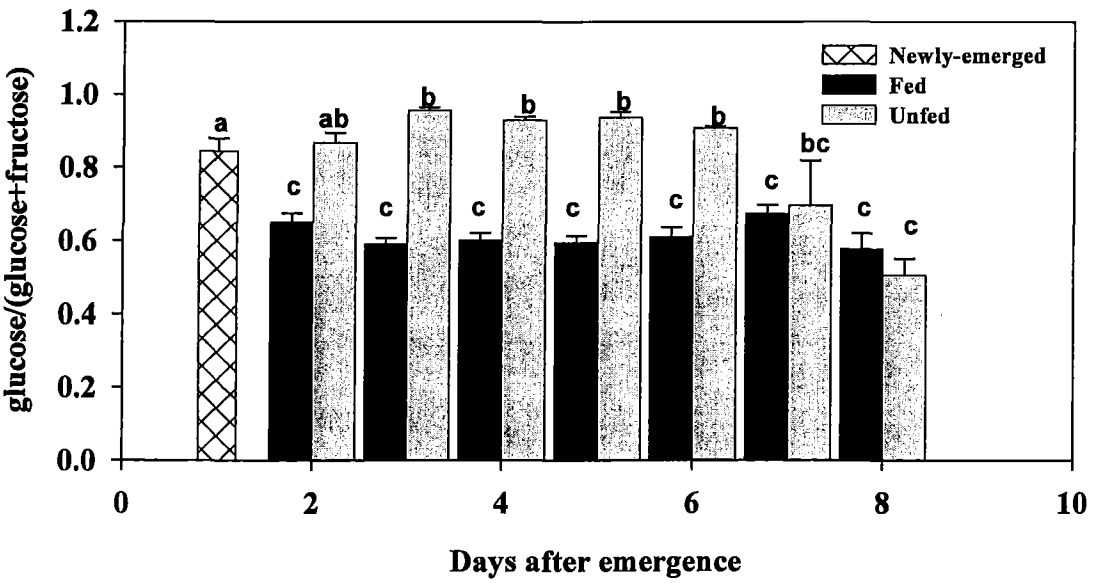

Figure 5.3: Comparison of the glucose/(glucose+fructose) ratio of newly emerged, fed and unfed parasitoids (for eight days to show significant differences). Bars sharing the same letter do not differ at $P = 0.05$. Error bars = +SE.

5.3.2 Fructose/total sugar (f/t) ratio in laboratory-reared parasitoids

The fructose/total sugar (f/t) ratio of newly emerged parasitoids was 0.1 ± 0.02 and this increased to 0.3 ± 0.03 after feeding for 24h. Thereafter, the f/t ratio remained high (range 0.25± 0.094 to 0.45± 0.021) in fed parasitoids, while the ratio ranged between 0.04± 0.01 and 0.08± 0.02 in unfed parasitoids (Figure 5.4).

The f/t ratio remained significantly different between fed and unfed parasitoids throughout their lifetimes ($P < 0.01$) (Figure 5.5).

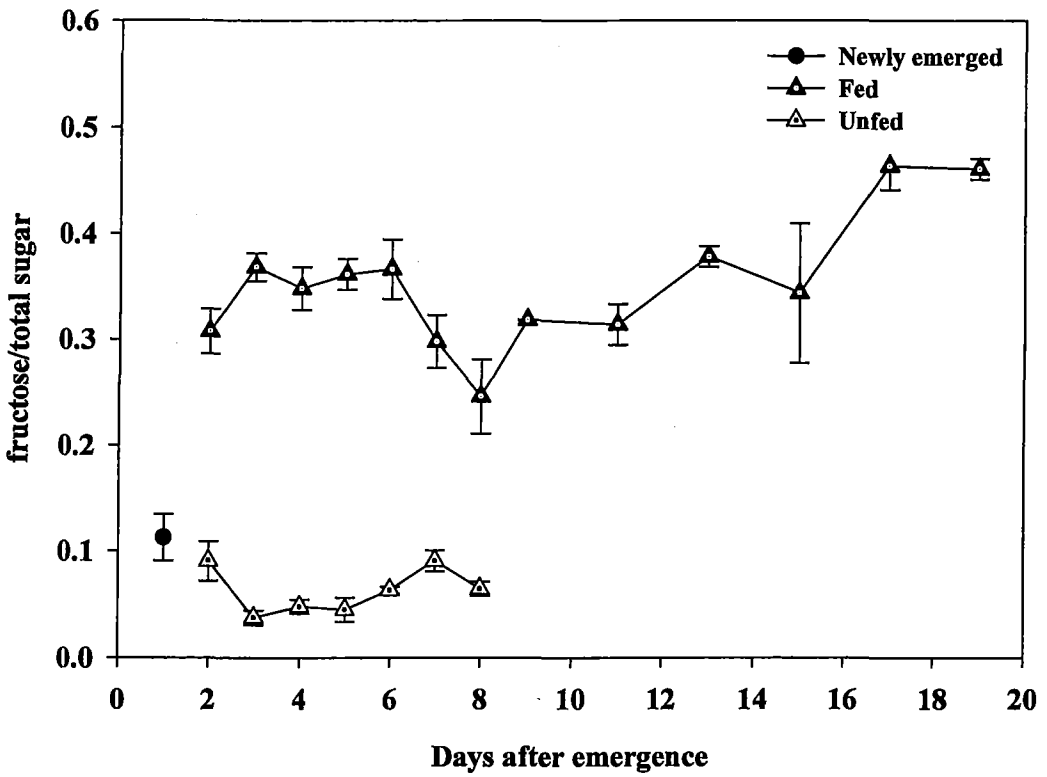

Figure 5.4: The fructose/total sugar ratio in newly emerged, fed and unfed parasitoids. The ratio increased in fed parasitoids with age and remained different from those of unfed parasitoids. Error bars = ±SE.

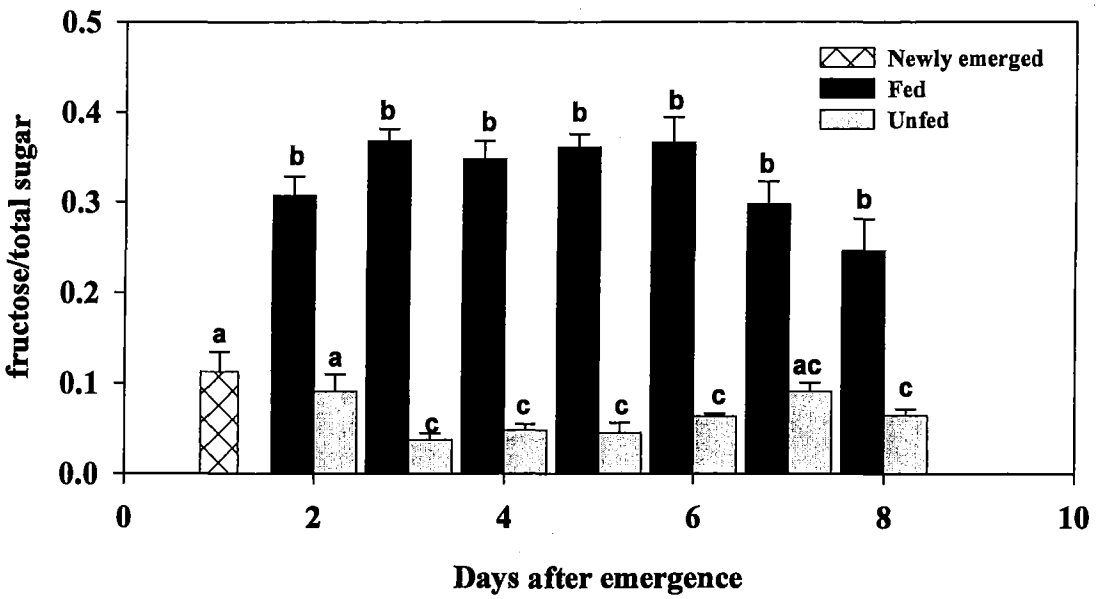

Figure 5.5: Comparison of fructose/total sugar ratio of newly emerged, fed and unfed parasitoids for eight days (to show significant differences). Bars sharing the same letter do not differ at $P = 0.05$. Error bars = +SE.

Tables 5.1 and 5.2 show maximum and minimum values of g/(g+f) and f/t ratios in newly emerged, fed and unfed parasitoids. The range of the g/(g+f) ratio was much wider compared with the f/t ratio in all three groups. There was no overlap between fed and unfed parasitoid f/t ratios, except that the maximum value for newly emerged and the minimum value for fed parasitoids overlapped slightly, by 0.01 (Table 5.2). Therefore, the threshold value for the f/t ratio, which separated fed and unfed parasitoids was 0.235, and was derived from the average of the maximum f/t ratio (0.24) detected in newly emerged and unfed parasitoids and the minimum f/t ratio (0.23) detected in fed parasitoids. The g/(g+f) ratios of all three groups overlapped (Table 5.1) and, therefore, could not be used to distinguish between these groups.

Table 5.1: The maximum and minimum values and the range (the difference between maximum and minimum ratio) of the glucose/(glucose+fructose) ratio in three different parasitoid groups.

Parasitoid group	Glucose/(glucose+fructose) ratio		
	Maximum	Minimum	Range
Newly emerged	0.97	0.58	0.39
Unfed	0.98	0.43	0.55
Fed	0.73	0.38	0.35

Table 5.2: The maximum and minimum values and the range (the difference between maximum and minimum ratio) of the fructose/total sugar ratio in three different parasitoid groups.

Parasitoid group	Fructose/total sugar ratio		
	Maximum	Minimum	Range
Newly emerged	0.24	0.02	0.21
Unfed	0.14	0.01	0.13
Fed	0.51	0.23	0.28

5.3.3 Sugar levels and f/t ratios in parasitoids collected near buckwheat plots

Table 5.3 shows the number of *M. hyperodae* adults collected at different distances from buckwheat plots. These parasitoids were classified as either fed or unfed based on their f/t ratios using the f/t threshold of 0.235.

Table 5.3: The number of parasitoids collected at different distances from buckwheat plots and the number and the percentage of parasitoids that showed the f/t ratio is greater than the threshold level.

Distance from buckwheat (m)	Total	f/t > 0.235	% > 0.235
0-2	19	15	79
2-4	9	6	66
4-6	3	2	66
6-8	5	0	0
8-10	3	0	0
10-12	0	0	-
12-14	0	0	-

Figure 5.6 shows the mean levels of glucose, fructose, sucrose and total sugars of parasitoids collected at different distances from buckwheat plots. The mean sucrose levels were high in

the first sampling area (0-2m). This was due to high sucrose levels in only six parasitoids out of 19 collected from that area. Fructose levels were high in the first three sampling areas. Glucose and total sugar levels were also higher in first three areas. The lowest levels of glucose and total sugars were recorded at 6-8m from the buckwheat and were slightly higher at 8-10m (Figure 5.6).

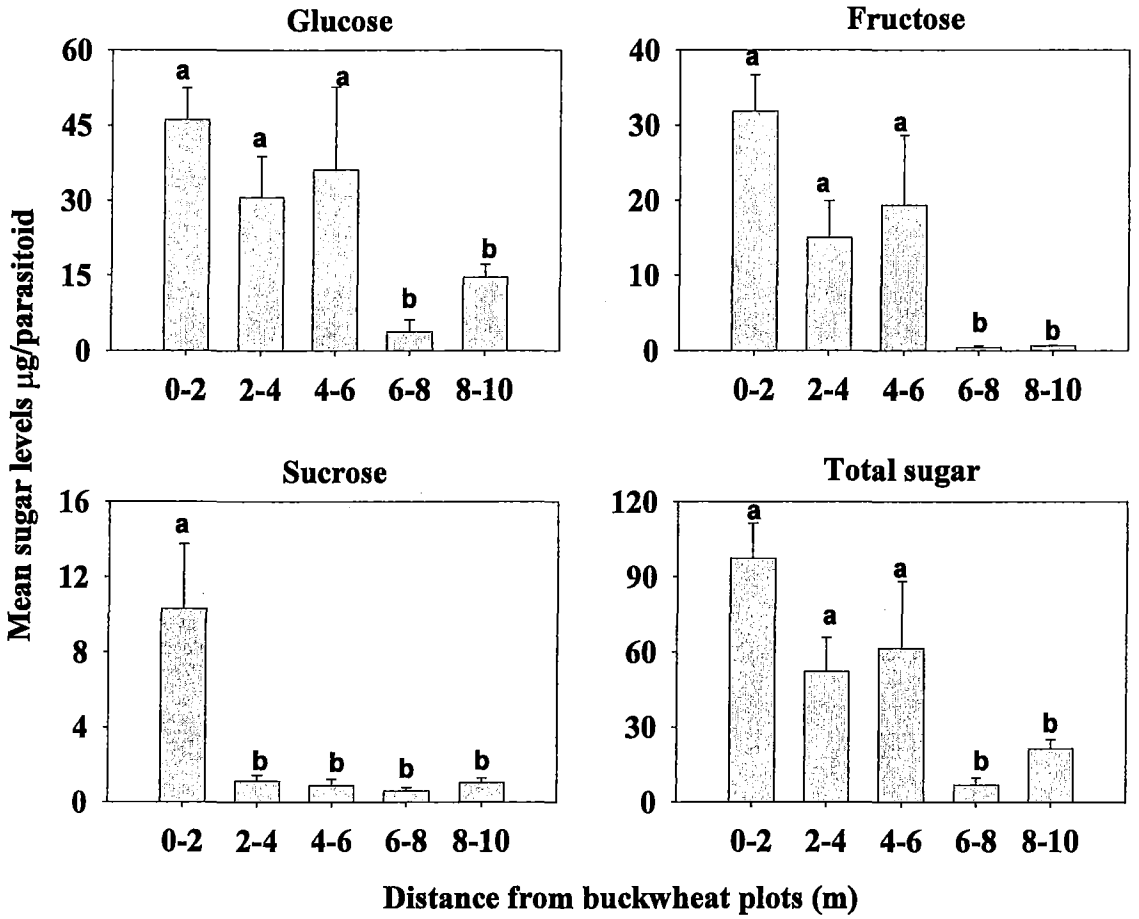

Figure 5.6: The mean 1) glucose, 2) fructose, 3) sucrose and 4) total sugar levels detected at different distances from buckwheat plots in the field. Bars sharing the same letter do not differ at $P = 0.05$. Error bars = +SE.

5.3.4 Fructose/total sugar ratios in parasitoids collected from road-side verges

Twenty nine *M. hyperodae* adults were collected from road-side verges near Lincoln. Six of them had a f/t ratio which was greater than 0.235. The f/t ratio for all other parasitoids was less than 0.17 (Table 5.4).

Table 5.4: The dominant sugars and the f/t ratios of the parasitoids collected from road-side verges near Lincoln. Shaded f/t ratios are above the threshold limits of unfed parasitoids.

Parasitoid number	Glucose	Fructose	Sucrose	Total	f/t ratio
1	3.25	0.36	0.41	4.02	0.09
2	4.05	1.17	1.15	8.48	0.14
3	30.38	20.63	5.13	71.28	0.29
4	5.75	1.77	1.90	13.08	0.14
5	4.74	0.48	0.50	8.69	0.06
6	1.89	0.38	0.68	4.51	0.08
7	8.53	2.14	0.60	14.59	0.15
8	2.27	0.38	0.45	4.90	0.08
9	2.25	0.42	0.46	4.47	0.09
10	5.20	0.33	0.29	7.74	0.04
11	25.42	14.79	5.37	52.45	0.28
12	24.14	4.22	1.04	37.17	0.11
13	14.03	1.21	1.52	18.42	0.07
14	14.17	9.32	5.73	32.33	0.29
15	10.36	7.48	0.56	20.27	0.37
16	10.09	0.55	1.19	15.41	0.04
17	6.33	0.36	0.96	10.31	0.03
18	10.00	5.60	0.77	18.84	0.30
19	3.36	0.57	1.27	7.41	0.08
20	7.84	0.70	12.00	23.76	0.03
21	9.21	0.42	0.85	13.19	0.03
22	5.60	0.40	1.05	8.93	0.05
23	2.87	1.04	0.30	6.09	0.17
24	5.33	0.49	0.59	9.43	0.05
25	7.13	0.35	0.39	21.53	0.02
26	4.30	0.20	0.22	6.85	0.03
27	11.57	0.86	1.00	18.43	0.05
28	48.04	27.45	3.05	86.34	0.32
29	1.78	0.29	0.43	4.27	0.07

5.4 Discussion

5.4.1 Sugar ratios of laboratory reared parasitoids

The g/(g+f) ratio cannot provide a reliable tool for discriminating between fed and unfed *M. hyperodae* because on days seven and eight, there were no significant differences ($P > 0.05$) between fed and unfed parasitoids (Figure 5.3). In contrast, the g/(g+f) ratio has been successfully used to discriminate fed from unfed *C. glomerata* (Steppuhn & Wäckers 2004). *C. glomerata* lived only three days with water, so comparison between fed and unfed parasitoids was possible only for these three days. In contrast, unfed *M. hyperodae* lived eight days with water. Therefore, comparisons were possible for eight days between fed and unfed *M. hyperodae*. It could be possible that the g/(g+f) ratio did not vary in the short lived unfed *C. glomerata* compared to the changes that occurred in *M. hyperodae* on days seven and eight.

Close to the time of death of unfed parasitoids, glucose levels rapidly declined (Figure 3.2, Chapter 3) compared with total sugars (Figure 3.1) and fructose (Figure 3.2), consequently altering the g/(g+f) ratio. As parasitoids approached death, they appeared only to metabolise glucose, rather than the greater range of sugars which they used earlier in their lives. It could be that a greater proportion of glucose compared with other sugars had been metabolised to release energy for survival. Probably the "threshold theory" of ageing (Clarke & Maynard 1961, Chapter 3) comes into play at this point and other sugars except glucose cannot be metabolised to satisfy the energy requirements needed by parasitoids approaching death. This possible 'ageing factor' therefore, prevents the use of the g/(g+f) ratio as a tool to distinguish between fed and unfed *M. hyperodae*.

The f/t ratio was always lower than 0.14 in unfed parasitoids and always higher than 0.23 in fed individuals and the differences between the f/t ratios of fed and unfed insects were always significant (Figure 5.5). This shows that the f/t ratio of *M. hyperodae* is a reliable tool to discriminate between fed and unfed parasitoids. Therefore, the f/t ratio can be used to reveal this insect's feeding history.

5.4.2 Dominant sugar levels in parasitoids collected near buckwheat plots

Fructose levels were high in the three collection areas closest to buckwheat (0-6m) compared with areas beyond 6m. This is again an indication that parasitoids feed from nectar in the field. For example, all insects studied by other workers for sugar levels using the anthrone test (van Handel 1985; Olson *et al.* 2000; Casas *et al.* 2005) and HPLC (Heimpel *et al.* 2004; Steppuhn & Wäckers 2004; Vattala *et al.* 2005) showed that fructose levels were significantly higher in fed compared with unfed individuals.

Sucrose levels were higher in parasitoids collected adjacent to buckwheat plots than in those collected further away. This is consistent with the findings in Section 3.3.4 of Chapter 3 which showed that parasitoids had high sucrose levels at least during the first hour after feeding from buckwheat. This strongly suggested that the parasitoids had fed from buckwheat, but the gut sugars had not been completely digested when the insects were captured. This could be verified by analysing parasitoid guts, rather than whole insects, immediately after capturing them from the field. Nectar analysis showed that buckwheat had high sucrose levels compared with other flowers (Chapter 2). High sucrose levels appeared to be reflected in the sugar analysis results of *M. hyperodae* shortly after feeding from buckwheat (Chapter 3). Therefore, the high sucrose levels recorded in the parasitoids collected near buckwheat plots could be used to indicate feeding from buckwheat in the field. It is important to freeze parasitoids immediately to stop sugar digestion in the gut to ascertain accurate results (Section 5.2.1). Higher sucrose levels in field-collected parasitoids near buckwheat plots could be a useful tool to confirm that nectar feeding had occurred shortly before the insect was captured.

5.4.3 An analytical tool to determine nutritional state and feeding history

The g/(g+f) ratio and the total sugar levels have been used together to examine the feeding history and nutritional state of *C. glomerata* (Steppuhn & Wäckers 2004). For *M. hyperodae*, Figures 5.7 and 5.8 show scatter plots for total sugar together with the g/(g+f) and the f/t ratios, respectively. The f/t ratio and total sugars together (Figure 5.8) show a more robust separation between fed and unfed parasitoids than the g/(g+f) ratio and total sugars together (Figure 5.7). The older (> 15 days) fed parasitoids plotted in Figure 5.7 fall closer to unfed parasitoids than that in Figure 5.8. Figure 5.8, therefore, provides a useful tool to discriminate unfed from older, fed parasitoids (>15 days).

Distinguishing fed from unfed parasitoids is easier when the fed ones have high total sugar levels. Then both those ratios become reliable because high (fed) and low (unfed) sugar levels place those two groups wider apart, as shown in both figures. In contrast, aged parasitoids had almost depleted their total sugars and, therefore, total sugar levels were quite similar to the unfed ones (Table 3.3, Chapter 3). Similar total sugar levels in fed and age is greater than 15 days and unfed *M. hyperodae*, including newly emerged individuals, made it difficult to discriminate between those parasitoid groups using the g/(g+f) ratio. Steppuhn & Wäckers (2004) analysed fed *C. glomerata* only up to 14 days. It is, therefore, interesting to see how reliable the g/(g+f) ratio and the total sugar levels are together as a tool, when parasitoids are approaching the death. *C. glomerata* lived more than 30 days with either sucrose, glucose or fructose solutions in the laboratory (Wäckers 2001).

Sugar levels of both unfed and fed *M. hyperodae* and *C. glomerata* were dominated by glucose, whereas fructose levels were low in unfed parasitoids and were high in fed individuals (Chapter 3, Steppuhn & Wäckers (2004)). Figure 3.2 in Chapter 3 shows that glucose metabolism was rapid in aged (> 15 days) *M. hyperodae* compared with fructose, whereas both sugars were utilised at similar rates when they were young. The g/(g+f) ratio is based on these two monosaccarides. Therefore the ratio variation observed between two groups of fed parasitoids, 1) age < 15 days and 2) age > 15 days resulted from the rapid glucose depletion in the latter group. The variation between these two groups of *M. hyperodae*, however, is not clearly visible in Figure 5.7. Therefore, the present study showed that the g/(g+f) ratio of *M. hyperodae* approaching death was not a reliable tool in discriminating fed from unfed individuals. In contrast, the difference is clear between these two groups in Figure 5.8.

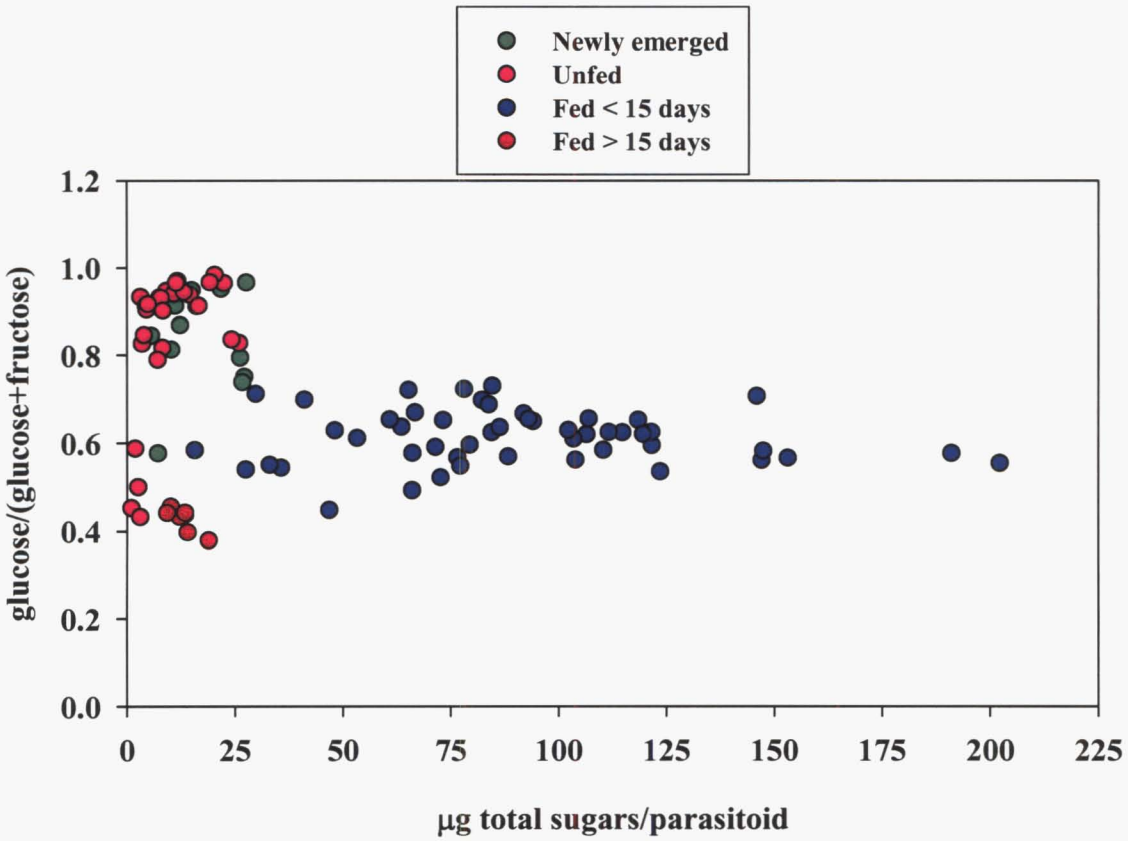

Figure 5.7: The total sugar levels (µg /parasitoid) vs the glucose/(glucose+fructose) ratio of every laboratory-reared individuals. a) newly emerged, b) unfed, c) fed and age is less than 15 days and d) fed and age is greater than 15 days

The f/t ratio is based on fructose and total sugar levels and both changed relatively uniformly throughout the life of *M. hyperodae*. In the early stages of their lifespan in both fed and unfed individuals fructose and total sugar levels changed at similar rates (Figure 3.3, Chapter 3). In the latter part of their lifespans (days seven and eight in unfed and days 17 to 19 in fed), total sugar levels declined rapidly compared with fructose levels. This was due to a rapid decline in glucose levels, the major fraction of the total sugars. The gradual decrease in fructose levels restricted the f/t ratio to a small range (Table 5.2). The small variation occurred within f/t ratios in fed and unfed parasitoids help to distinguish between these two groups. Therefore, the f/t ratio provides a much better tool than the g/(g+f) ratio to disclose the feeding history of *M. hyperodae*. The analytical tool, in combination with the f/t ratio and total sugar level to discriminate between fed and unfed *M. hyperodae*, is presented in Figure 5.8. Any parasitoids above this threshold, therefore, had fed at some stage, whereas those below the threshold had not. The line separating the total sugar levels of parasitoids was derived from the maximum total sugar level (27.6µg) of unfed parasitoids, including those newly emerged (Figure 5.8). The line in Figure 5.8 separates the parasitoids in relation to high and low total sugar levels. This line shows the maximum total sugar level (27.6µg) that was detected from pooled sugar

data of newly emerged and unfed parasitoids (i.e., never fed during their life time). The total sugar threshold can be used to understand the availability of sugar levels in those individuals, which is also an indicator of the parasitoid fitness assuming that the parasitoids are time limited and not subject to predation and other sources of mortality. A high level of body sugars would help parasitoids live longer and hence they have more time to lay their eggs. In contrast, low nutrient levels would result in a shorter lifetime. The aged parasitoids also had low level of sugars and those parasitoids, therefore, occurred closer to unfed parasitoids in Figure 5.7 since they had almost completed their life and utilised most of their body sugars (Figure 3.1, Chapter 3). Those parasitoids, however, occur above the f/t ratio threshold limit (Figure 5.8), because they had higher f/t ratios compared with unfed ones. Those parasitoids which never had any sugar meals occurred closer to aged individuals, but were well below the f/t ratio threshold. The tool presented in Figure 5.8 is, therefore, capable of providing all this information for an individual parasitoid. This information can be used to discriminate fed from unfed as well as fed-young from fed-aged *M. hyperodae*.

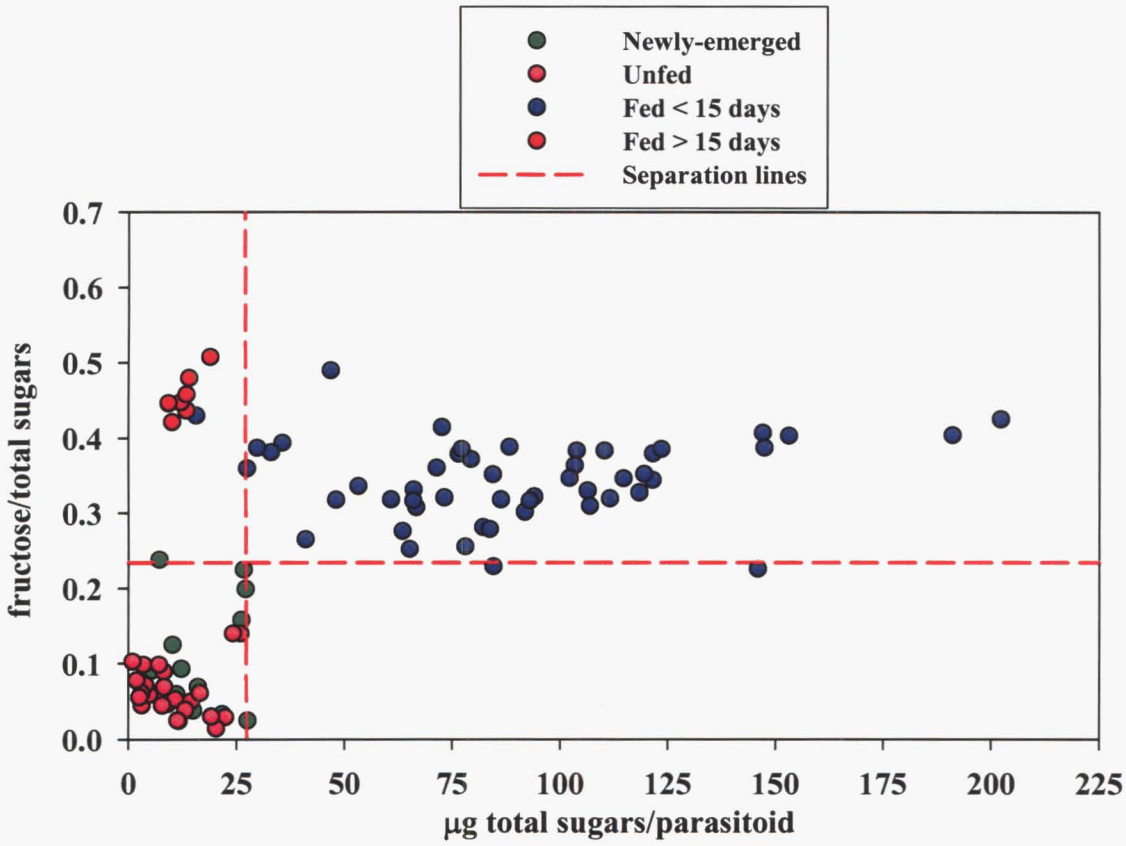

Figure 5.8: The total sugar levels (µg /parasitoid) vs the fructose/total sugar ratio of every laboratory reared parasitoids. a) newly emerged, b) unfed, c) fed and age is less than 15 days and d) fed and age is greater than 15 days. Dashed lines are drawn at x = 0.235 (average of the maximum f/t ratio (0.24) detected in newly emerged and unfed parasitoids and the minimum f/t ratio detected in fed parasitoids) and y = 27.6µg (The maximum total sugar level detected from pooled sugar data of newly emerged and unfed parasitoids (i.e., never fed during their lifetime))

5.4.4 Application of the discrimination tool to field collected parasitoids

The f/t ratio vs total sugar levels of field-collected parasitoids are presented in Figure 5.9. The thresholds derived for the f/t ratio and total sugars shown in Figure 5.8 were used to separate field collected parasitoids into different groups. Seventy nine percent of parasitoids collected from within 2m of buckwheat plots had high f/t ratios and, hence, must have fed from buckwheat. Twenty three out of 31 *M. hyperodae* collected from within 6m of the buckwheat plots (Table 5.3) were above the f/t threshold indicating that they had fed. All parasitoids captured from 6-10m from the buckwheat were below the f/t threshold indicating that they had not fed. This strongly suggests that parasitoids do feed from buckwheat nectar in the field and that they disperse up to 6m away from the buckwheat after feeding. The absence of any fed parasitoids beyond 6m from the buckwheat also strongly suggests no sugar sources other than buckwheat were available in the field sites. These insights illustrate the utility of the f/t ratio for understanding parasitoid feeding ecology. The f/t ratio also provides insights about the distance parasitoids travel after feeding. It may be possible to use the f/t ratio as an insect 'marker' to help to understand parasitoid movement in relation to nectar sources in the field.

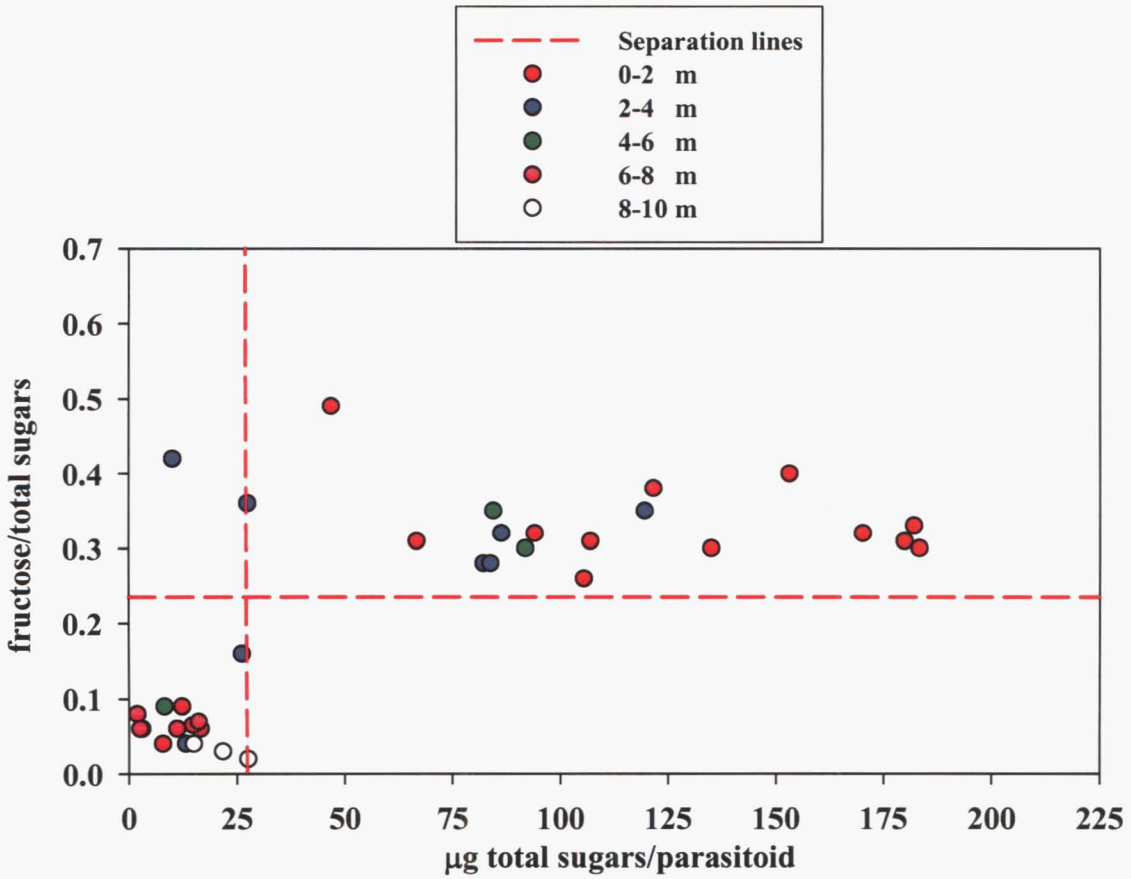

Figure 5.9: the total sugar levels (µg /parasitoid) vs the fructose/total sugar level ratio of every field collected individuals at different distances from buckwheat plots, a) 0-2m, b) 2-4m, c) 4-6m, d) 6-8m and e) 8-10m. The dashed lines were adopted from Figure 5.8.

Figure 5.10 shows that six out of 29 parasitoids captured from road-side verges had fed on sugars (Figure 5.10). The source of this sugar is unknown. It is a possible that *M. hyperodae* had fed from some flowering weeds, which are abundant along road-side verges, as well as along fence lines. These species included *Taraxacum* spp., *Capsella* spp., *Stellaria* spp and *Veronica* spp. However, the sugar sources available to *M. hyperodae* in pasture are likely to be scarce, as it cannot use clover flowers (Chapter 2). The nectar accessed by *M. hyperodae* on road-side verges does not appear to occur in pasture since parasitoids collected from 6m away from buckwheat did not show any evidence of having fed (Section 5.3.2).

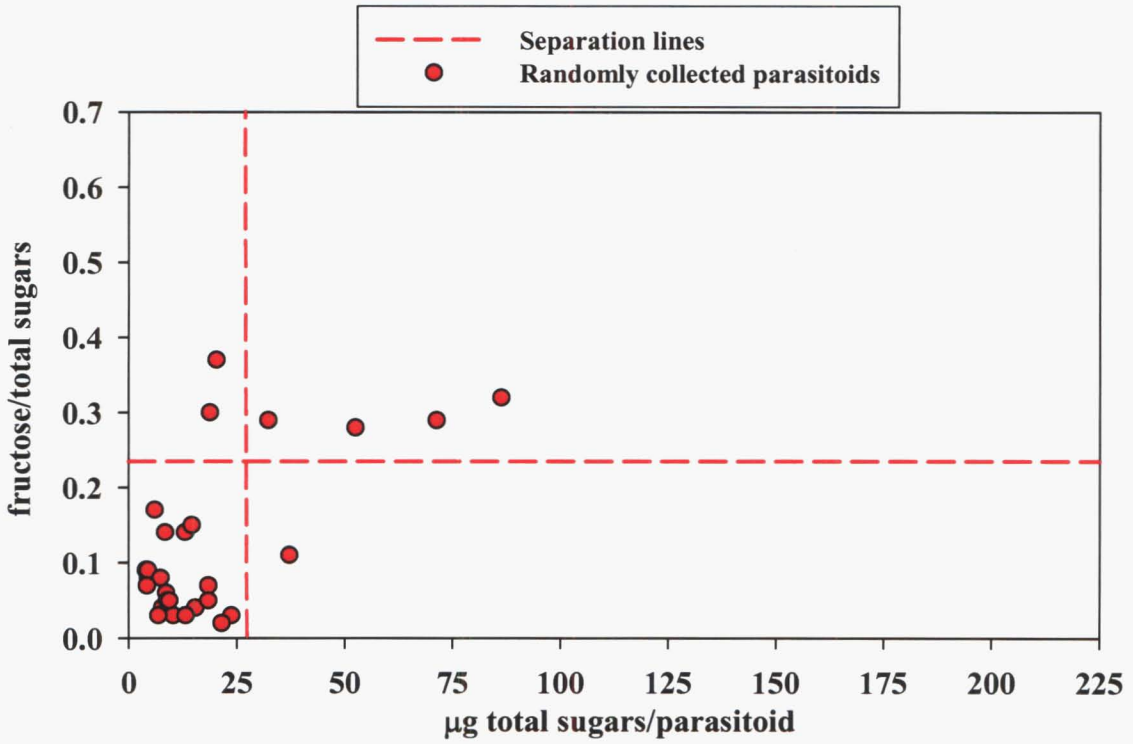

Figure 5.10: Total sugar levels (µg /parasitoid) vs fructose/total sugar level ratio of 29 parasitoids collected from road-side verges. The dashed lines were adopted from Figure 5.8.

5.5 Conclusions

M. hyperodae is time limited during November and December and increasing its longevity could increase its fecundity during this period (Phillips *et al.* 1998). Higher sugar levels in parasitoids collected from near buckwheat plots strongly suggested these parasitoids had fed from buckwheat. Increased sugar levels could increase parasitoid longevity. Assuming sugar availability limits *M. hyperodae* longevity, access to buckwheat could increase *M. hyperodae* fecundity. The research described in this chapter has, therefore, elucidated one of the links between the provision of nectar sources in the field and parasitoids 'fitness'. It remains to be shown that the potential for increased parasitoid longevity conferred by the provision of buckwheat flowers can be manifested as increased parasitism rates of *L. bonariensis*. This is the main aim of Chapter 6.

Some studies showed that a link between parasitoids' body sugars and 'signature sugars', such as sugars that can be detected only in honeydew (Wäckers *et al.* 2005). There is no study to date that has shown a direct link between a specific nectar and sugar content in the parasitoid body. Chapter 3 showed that parasitoids had high sucrose levels immediately after

feeding from buckwheat and Chapter 2 has already shown that buckwheat nectar had a high proportion of sucrose compared with some other nectar sources. This aspect needs to be further investigated in relation to parasitoids feeding in the field. Findings from such investigations would add weight to the promotion of CBC as a pest management tool. A linkage with higher parasitism rates in the field is still required, and measuring pests' parasitism rates in relation to buckwheat plots is the main aim of Chapter 6.

It appears that *M. hyperodae* dispersed less than 6m after feeding from buckwheat. Therefore the analytical tools developed in this chapter may be useful in understanding parasitoid movements in addition to their feeding history and the nutritional state. Separate research programmes that specifically focus on parasitoids' movements are also needed. This can be done by using insect markers such as rubidium chloride (Hagler & Jackson 2001; Lavandero *et al.* in press-b). There is also research under way to use an antioxidant in buckwheat nectar as an insect marker (S. D. Wratten, pers. comm).

Plate 5.1: Suction sampler used to collect parasitoids.

Plate 5.2: Mesh bag placed in the intake pipe of the suction sampler to trap insects.

CHAPTER 6

PARASITISM RATES OF *Listronotus bonariensis* IN THE PRESENCE AND ABSENCE OF BUCKWHEAT

6. 1 Introduction

Suppressing pest numbers to below economic thresholds is the aim of biological control. The effects of 'resource subsidies' provided to natural enemies on prey suppression (Kean *et al.* 2003; Tylianakis *et al.* 2004; Eubanks & Styrsky 2005; Gurr *et al.* 2005) in agricultural landscapes have not yet been comprehensively studied. A few studies have shown that the provision of floral resources can suppress pest populations (e.g., White *et al.* 1995; Hickman & Wratten 1996; Patt *et al.* 1999), but none of these conservation biological control (CBC) programme has shown economic benefits resulting from the reduced pest populations.

Parasitism rate (parasitised hosts per total number of hosts) has been widely used as an indicator of parasitoid efficacy (Mitchell *et al.* 1997; Cappuccino *et al.* 1998; Haseeb *et al.* 2000), thus, demonstrating that floral resources can increase parasitism rates is useful for assessing the success of CBC programmes. However, there are some situations in which parasitism rates may not provide a robust indicator of pest suppression due to peculiarities of some parasitoid/host systems (van Driesche & Bellows 1996). In those situations, which are beyond manipulation in the field, parasitism rate still remains a useful method of measuring parasitoid efficacy. Rates of parasitism of *L. bonariensis* have been used to measure the efficacy of *M. hyperodae* in New Zealand (Goldson *et al.* 1998b). However, the ultimate measure of efficacy must be pest population reduction.

Increases in parasitism rates associated with the provision of flowers have been recorded in several field studies. Higher parasitism rates of the alfalfa caterpillar, *Colias philodice* Boisduval (Lepidoptera: Pieridae), were recorded in alfalfa fields adjacent to flowering weeds compared with fields more distant from the flowering weeds (Allen & Smith 1958). Parasitism rates of the tent caterpillar, *Malacosoma americanum* (F.) (Lepidoptera: Lasiocampidae), were increased 18-fold in orchards with understorey wild flowers (Leius 1967). Parasitism of the gram pod borer, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), was increased four-fold in a chickpea crop bordered by flowering coriander plants

(Pimbert & Srivastava 1989). Parasitism of the potato moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), was greater within 20m of flower strips than further away (Baggen & Gurr 1998). Parasitism rates decreased exponentially with the increased distance from floral resources for the aphid, *Metopolophium dirhodum* Mordvilko (Aphidinae: Macrosiphini), in barley fields (Tylianakis *et al.* 2004).

The possibility of increasing parasitism of *L. bonariensis* in mid summer by increasing the efficacy of *M. hyperodae* during early summer was suggested by Phillips *et al.* (1998). *M. hyperodae* is time limited from mid November to late December, then it becomes egg limited for the rest of the summer (Phillips, unpublished). Therefore, it appears that the provision of buckwheat could influence the efficacy of *M. hyperodae* for a limited period during mid November to late December (Figure 1.2, Chapter 1). Although there have been many examples where floral resources have been provided to increase the efficacy of natural enemies in orchards, cereals and vegetable crops, this concept has never previously been applied to pastures. This chapter describes an experiment designed to measure parasitism rates of *L. bonariensis* in pasture with and without flowering buckwheat plants. The previous two chapters have shown that the provision of buckwheat in the field increased parasitoid abundance in the vicinity of buckwheat plots, and parasitoids have benefited from nectar sources. The aim of this chapter is to measure the effect of sowing buckwheat in pastures on parasitism of *L. bonariensis*.

6.2 Materials and methods

6.2.1 Experimental design

A private, irrigated farm was identified by Wrightson Research for conducting this experiment. Mostyn Farm is situated near Leeston Road about 2 km towards Leeston from Springston Junction, Canterbury, New Zealand. The experiment was conducted from mid September 2004 to April 2005. The two paddocks chosen for the experiment were sown with non-endophyte perennial ryegrass (*L. perenne*). The two paddocks were approximately 500m apart. One paddock was larger than the other and this was divided into four blocks while the smaller one was divided into two (Figure 6.1). Buckwheat plots were arranged to avoid locating two plots next to each other (Figure 6.1). One experimental block comprised ryegrass with two treatments, plus buckwheat and minus buckwheat. The gap between these two treatments was 30m. Buckwheat plots were placed 1m away from the fence line. The size of

each buckwheat plot was 10 x 2m. Areas from which *L. bonariensis* was sampled were marked on the grass in front of the plus and minus buckwheat plots, leaving a 0.5m gap between treatment plots and sampling areas. The size of these sampling areas was also 10 x 2m. The sampling areas near the buckwheat treatment (near buckwheat) and without buckwheat (control) were, therefore, located 3.5m into the paddock from the fence and were 30m apart. This is well beyond the 8m distance to which buckwheat influenced the abundance of *M. hyperodae* in the field (Chapters 4 and 5), so the six blocks were considered independent for the purpose of this experiment. However, the sampling areas located near buckwheat were well within the range of influence of buckwheat flowers on *M. hyperodae* (Chapters 4 and 5).

The buckwheat plots were cultivated and sown in late September 2004. Sowing dates were chosen based on data in Bowie *et al.* (1995) so that the buckwheat would be flowering by mid November to synchronise with the emergence of *M. hyperodae* adults from the overwintered generation (Figure 1.2, Chapter 1). Two further sowings were made in November and January to maintain the availability of flowers. The buckwheat and control plots were watered, as required, to maintain healthy plants throughout the sampling period. All weeds and other flowering plants were manually removed, both from the buckwheat plots and from the pasture up to 30m from the buckwheat and the control plots. To avoid damage to the buckwheat from livestock, electric fencing was erected around the plots.

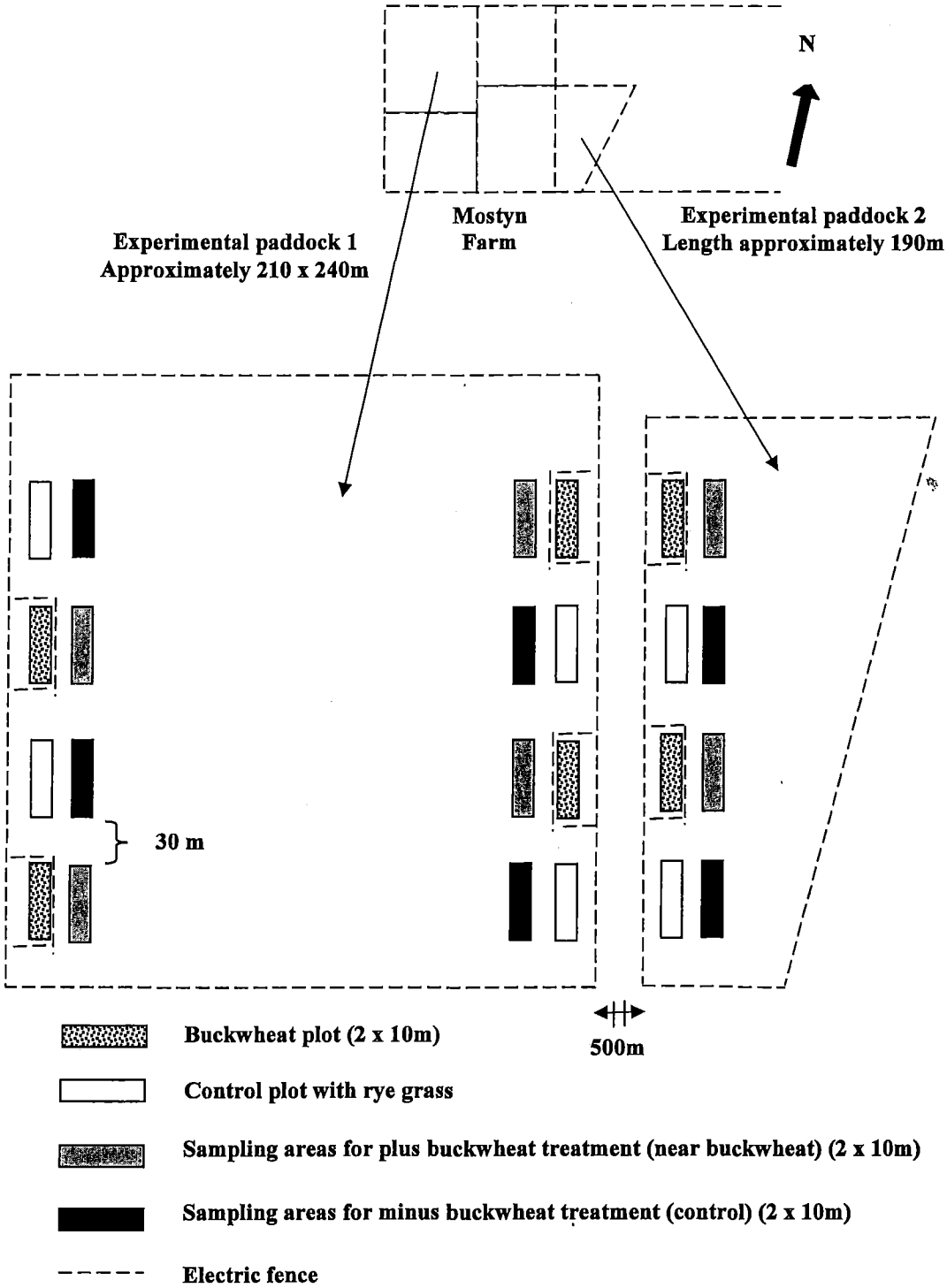

Figure 6.1: The two ryegrass paddocks used to sample weevils from mid November 2004 to end of April 2005. The two experimental paddocks were approximately 500m apart. Both paddocks were completely covered with ryegrass except for areas covered with buckwheat. The figure is not to scale.

6.2.2 *L. bonariensis* collection and dissection

L. bonariensis sampling began on 17 November 2004 and continued weekly until 29 April 2005 (n = 24). Maintaining a strict one week interval between consecutive collections was not always possible due to rain since dampness seriously affected weevil collection efficiency. Therefore weevil collecting occurred on dry days, and the number of days between consecutive collections varied.

A suction sampler (Plate 5.1, Chapter 5) was used to collect *L. bonariensis*. All materials collected in the mesh bag (Plate 5.2, Chapter 5) were transferred to a labelled bag to sort and separate *L. bonariensis* in the laboratory. All collections were labelled with the date and collection area. Weevils were dissected under 50X magnification to estimate parasitism rates.

6.2.3 Statistical analysis

Data for weevil numbers were analysed using generalised linear model (GLM) for poisson distribution with log link and parasitism rates for each collection date from the two treatments were analysed using GLM for binormial distribution with a logit link (GenStat V.7).

6.3 Results

The two buckwheat plots in the small paddock failed to produce healthy plants due to poor soil conditions. Water-logging was frequent, especially after irrigation, which occurred twice weekly. As a result, the buckwheat plants either died or did not grow well. Several attempts were made to re-sow the buckwheat, but these were unsuccessful. There were a few scattered plants remaining, but this density was not comparable with the other four buckwheat plots in the larger paddock. Therefore, the two blocks with these buckwheat plots were eliminated from the statistical analysis and only the data obtained from the four blocks in the larger paddock were used.

The number of weevils captured remained low until the third week of December 2004. The mean number of weevils per collection date varied from 1.0 ± 0.41 (± SE) to 4.0 ± 1.58 (± SE) near buckwheat, while it ranged from 0.8 ± 0.48 to 3.8 ± 0.48 in the controls. The first summer generation of adult weevils began emerging in the last week of December 2004. The

highest numbers were recorded in the last week of January 2005 and were 26.0 ± 5.82 and 20.3 ± 3.71, near the buckwheat and controls, respectively (Table 6.1). There were no significant differences between the numbers of weevils captured ($P > 0.05$) near buckwheat and controls throughout the sampling period (Figure 6.2).

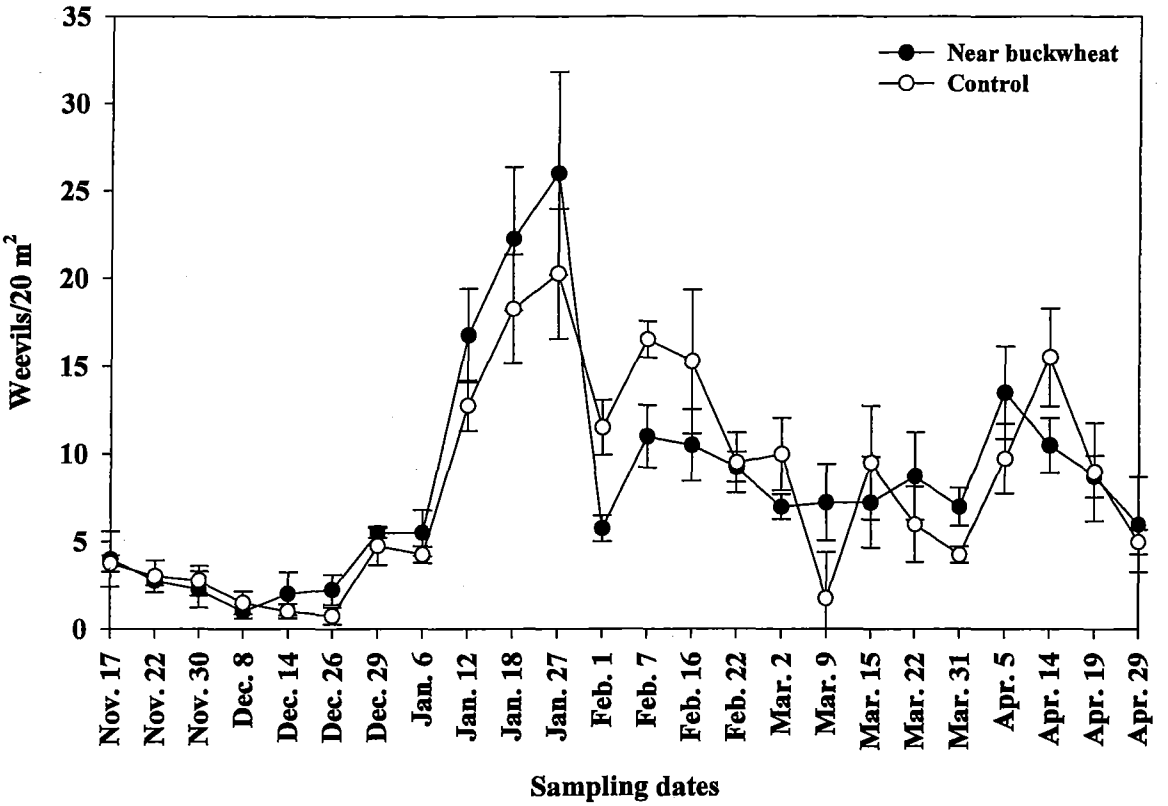

Figure 6.2: The mean number of weevils captured at the two sampling areas at Mostyn Farm. There were no significant differences between two treatments for all 24 days ($P > 0.05$). Error bars = ± SE.

Parasitism rates of *L. bonariensis* showed no significant differences between near buckwheat plots and controls from 17 November to 8 December ($P > 0.05$). The differences observed on 8, 14 and 26 December could not be reliably tested, because zero parasitism rates were recorded near buckwheat plots on 8 December and control plots on 14 and 26 December. On these dates, the numbers of weevils captured were very low so the chances of sampling parasitised weevils were also low. However, parasitism rates were significantly higher ($P < 0.01$) near buckwheat plots than in control plots from the last week of December until the second week of February (Figure 6.3). Parasitism rates near buckwheat plots were more than double those of control plots during this period. Parasitism rates in control plots began to increase from the second week of February and reached similar levels to those near

buckwheat plots in the last week of February. The levels in both sampling areas remained high until the second week of April and did not significantly differ ($P > 0.05$) during this period (Figure 6.3).

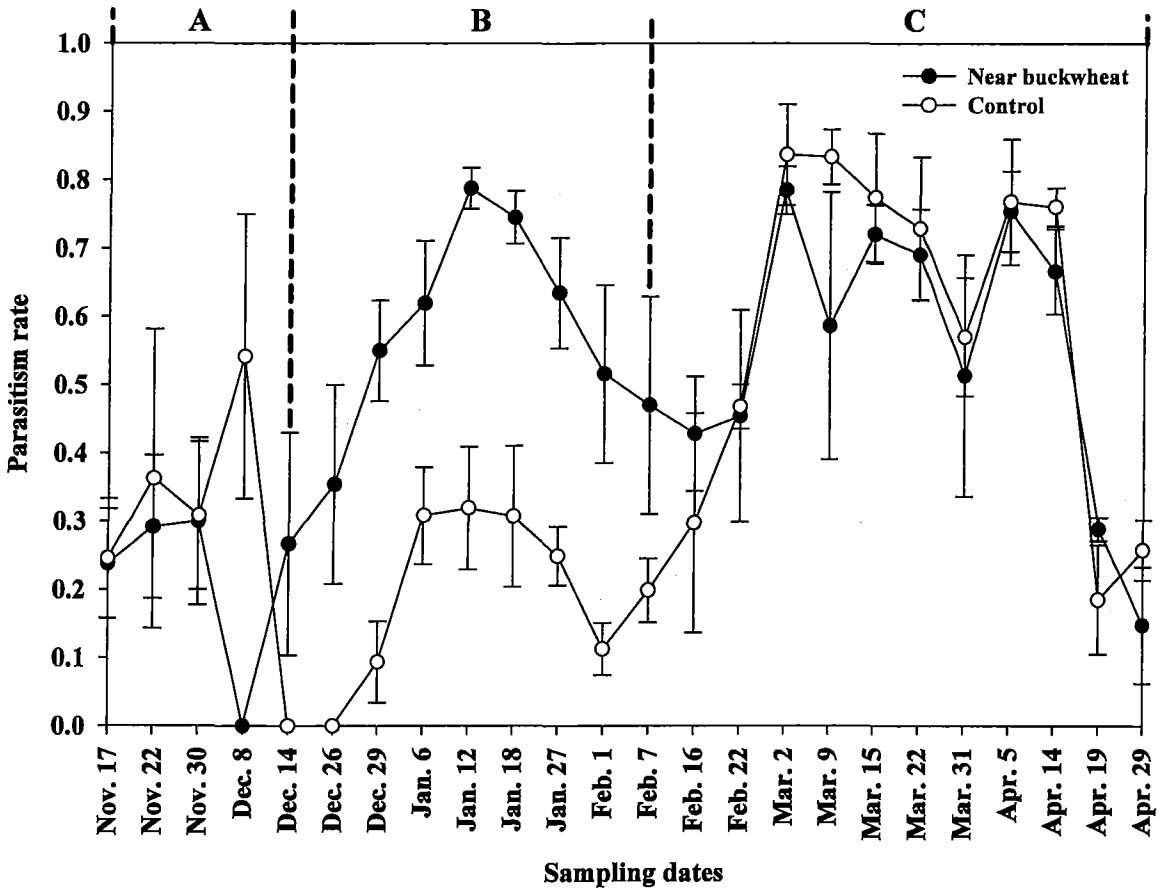

Figure 6.3: The mean parasitism rates (*M. hyperodae* egg or larva/number of weevil captured) at two sampling areas at Mostyn Farm. In periods A and C there were no significant differences between treatments ($P > 0.05$) while in period B there were significant differences between treatments ($P < 0.01$). Error bars = ± SE.

Figure 6.4 shows the mean number (density) of weevils captured, and the mean number of *M. hyperodae* eggs or larvae detected in them. Although, there were no significant differences in adult weevil densities between the two sampling areas, the density of adult weevils began to increase earlier near buckwheat plots than in the control plots, with the first increases noted in the second week of December and last week of December, respectively.

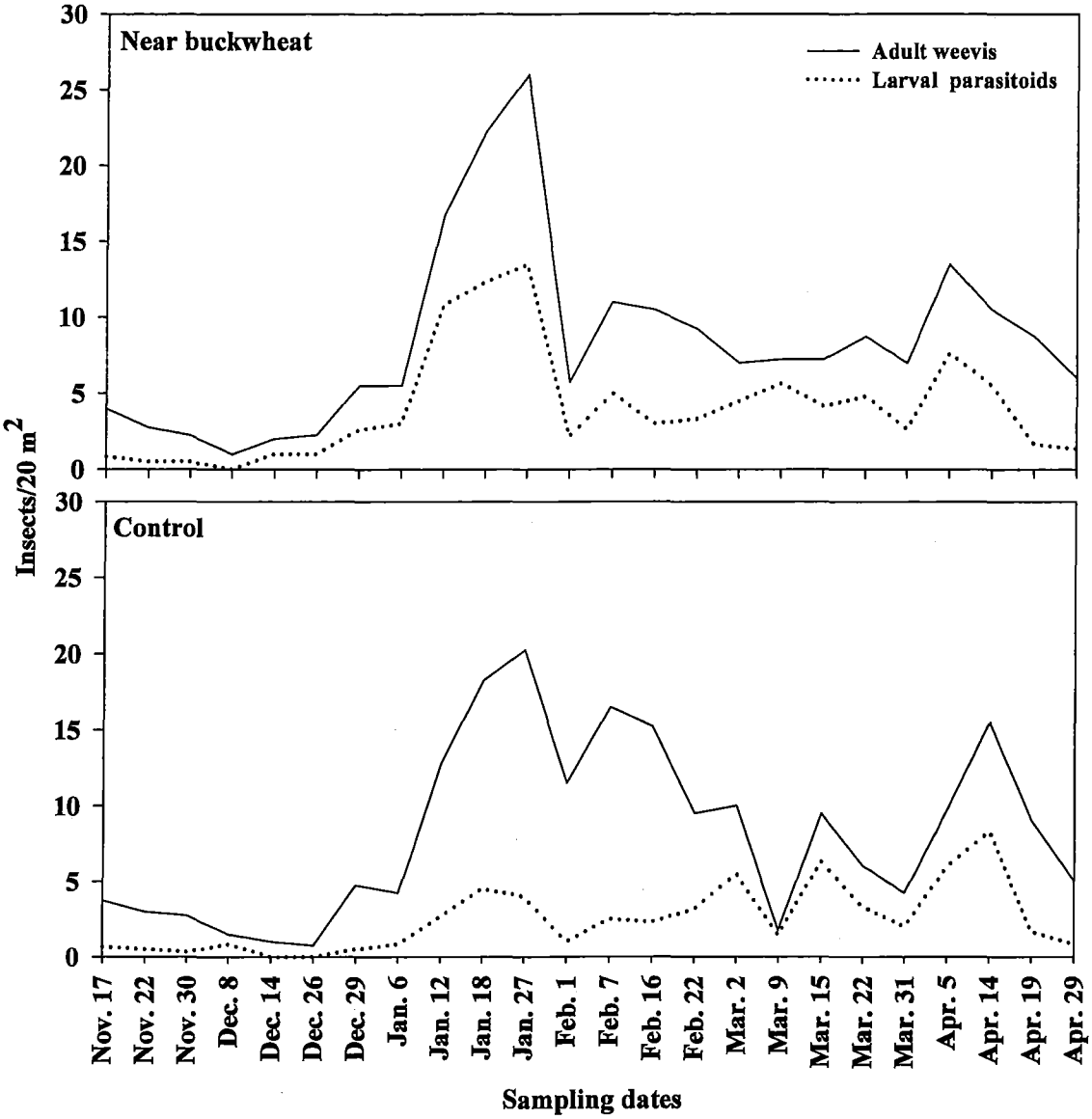

Figure 6.4: Densities of adult weevils and larval parasitoids near buckwheat and control plots.

6.4 Discussion

The scarcity of adult weevils observed in the present experiment during this period was consistent with previous studies which showed that the first summer generation of adult *L. bonariensis* began to emerge from the second week of December in the field and peaked in January (Goldson *et al.* 1998a; Phillips *et al.* 1998). Phillips *et al*'s 1998 study was conducted for eight months, from September 1996 to April 1997, but the study by Goldson *et al.* (1998a) was for five years, from 1990 to 1995. In the latter study, although the dates of peak *L. bonariensis* adult populations were varied from one season to the next, in 1995, the peak was

reported on 9 January. *L. bonariensis* and *M. hyperodae* also showed similar population dynamics in a five-year study conducted between 1996 and 2000 at the same site at the Lincoln AgResearch Farm (Phillips, unpublished). In the present study, the first summer generation of *L. bonariensis* adults also began to emerge in the second week of December near buckwheat plots, although this appeared to occur two weeks later in control plots. However, there were no significant differences in weevil numbers between the two sampling areas, so this variation was probably random.

The temporal variations in population densities of *L. bonariensis* adults and *M. hyperodae* observed in the present experiment were consistent with those seen in previous studies (Goldson 1998b; Phillips *et al.* 1998). However, much lower densities of insects were recorded in the current experiment. Turf sampling methods were used in addition to suction sampling in the previous studies (Goldson *et al.* 1998b; Phillips *et al.* 1998), which collected a greater number of weevils from the pasture. For example, Phillips *et al* (1998) showed that more than 150 weevils per m^2 were collected in mid January 1997 (Figure 1.2, Chapter 1). Although, the suction sampler used in this study collected little more than 25 weevils per $20m^2$ during the same period, the expected weevil densities were much higher because Proffitt *et al.* (1993) showed that there were no significant differences in the number of weevils collected from these two sampling methods. Therefore, it is possible that the *L. bonariensis* population has been declined over the years as *M. hyperodae* become more widely distributed in Canterbury. It is also possible that lower population densities occurred at the experimental paddocks during the present study, as high levels of year-to-year fluctuation in weevil densities were observed previously on the AgResearch Farm at Lincoln (Goldson *et al.* 1998a; Phillips unpublished). However, it appears that the patterns of variation in population densities of adult weevils and larval parasitoids were similar, irrespective of the sampling methods used.

The increased parasitism rates of the first summer generation of adult weevils suggested that the provision of floral resources increased the efficacy of *M. hyperodae* and, as a result, parasitoids were able to parasitise more weevils near buckwheat compared with controls. The potential to increase the efficacy of *M. hyperodae* during the November – December period was previously suggested (Phillips *et al.* 1998). Phillips *et al.* (1998) showed that overwintered parasitoids laid only 15% of their egg load from mid November to mid December, due to the scarcity of hosts during this period. They suggested one way of

overcoming the scarcity of weevils would be to increase the longevity of *M. hyperodae* until adult weevils become abundant in the field. Then the parasitism would increase because more adult parasitoids would be present when adult weevils become abundant. Laboratory experiments showed that buckwheat feeding increased the longevity of *M. hyperodae* (Chapter 2). Chapter 5 showed that parasitoids had increased their body sugar levels in the field near buckwheat plots indicating that they had fed from buckwheat. Higher parasitism rates observed near buckwheat suggested that parasitoids in the vicinity of buckwheat appeared to have laid more eggs. This could be due to both, increased parasitoid longevity from buckwheat feeding, and due to buckwheat attracting parasitoids (Chapter 4) from the surrounding pasture. The latter group are also likely to be benefited from buckwheat and increased their longevity. Therefore, the provision of floral resources appeared to increase the longevity of *M. hyperodae* resulting in increased parasitism rates. However, the results did not show that adult weevil densities were suppressed near buckwheat plots. It is possible that weevil movements to and from sampling areas prevented any demonstration of this effect.

The differences between parasitism rates observed near buckwheat and in control plots could be used to calculate the percentage of adult parasitoids surviving due to the provision of floral resources, assuming increased parasitism was due to increase parasitoid longevity rather than the attraction of parasitoids to buckwheat from surrounding areas. Phillips *et al* (1998) estimated that a 20% increase in parasitoid density could increase the parasitism rates by 31% in January. Their estimates were based on following calculations:

- Number of adult parasitoids survived form overwintered larvae = 1.8/m²
- Parasitoids realized fecundity = 44 eggs
- Number of larval parasitoids observed on 20 January = 51 larvae/ m²
- So that, a 20% increase in survival of parasitoids should increase 20/100 x 44 x 1.8 of additional larvae in the first generation of *M. hyperodae*, and = 16 larvae
- Therefore, increase in parasitism rate = 16/51 x100%
  = 31%

In the present study, the difference in parasitism rates between near buckwheat and control plots was 40% in January 2005. This figure can be used to reverse the calculation of Phillips *et al.* (1998) to estimate the increase in parasitoid densities near buckwheat plots.

- Increased number of larvae based on 40% parasitism rate = 51 x 40/100
  = 20.4 larvae
- Let the increased survival percentage of overwintered parasitoid = a
- Then, a x 1.8 x 44 = 20.4
  a = 20.4/1.8. x 44
  a = 26%

Therefore, the provision of floral resources in pasture increased *M. hyperodae* adults density by 26%. Based on estimates of Phillips *et al* (1998) and findings of the present study it seems that 2.3 parasitoids per m^2 were present near buckwheat plots while 1.8 parasitoids per m^2 were present in the controls. However these figures are based on the assumption that the all assumptions made by Phillips *et al* (1998). are still valid for this study. Although the patterns of variation in population densities of adult weevils and larval parasitoids were similar with previous studies, the present study showed that the weevil densities were much lower compared with those studies. Therefore, this estimate is very approximate, but serves to indicate the magnitude of the effects.

6.5 Conclusions

This study demonstrates that the fourth step (higher parasitism rates) of CBC hierarchical levels (Section 1.6, Chapter 1) has been achieved as a result of provision of floral resources in the field. These findings are useful in understanding the effects of the provision of floral resources in pasture.

There were many limitations encountered in selecting, planning and implementing this experiment. First, convincing farmers to allocate some.productive pasture land for buckwheat growing was not easy. Second, selection of appropriate pasture fields with irrigation and non-endophyte ryegrass that is around two years of age was also difficult. Third, protecting buckwheat plants from livestock also made the experiment even more difficult to conduct. If some suitable land could be allocated exclusively to conduct the same experiment then in addition to the parasitism rates, it could be possible to obtain quantifiable data on pest suppression as well. This is because a higher proportion of the land could be devoted to buckwheat and sampling from larger areas could produce more information. More flowering plants species should also be included in such an experiment to identify those that are more acceptable to farmers. Testing of native flowers for their suitability for CBC programmes

should be given a priority. Collating information on flower feeding of native *Microctonus* spp. in New Zealand (Shaw 1993) would probably be a good starting point for this research.

CHAPTER 7

GENERAL DISCUSSION

The science of classical biological control (Classical BC) of arthropods by arthropods has had more than 100 years of history (Greathead & Greathead 1992; Radcliffe & Flanders 1998; Gurr & Wratten 2000). Biological control statistics show that 40% of introduced arthropod biological control agents established in their new environment, but 75% of those established were not effective in controlling pests. Its success has been consistently around 10% over this period (Gurr & Wratten 2000).

In addition to the low success rate, there has been increasing concern over the adverse effects of introduced biological control agents on non-target species, such as native species (Pimentel *et al.* 1984; Goldson & Phillips 1990; Barratt *et al.* 1997; Lynch & Thomas 2000; Louda *et al.* 2003; Stiling & Simberloff 2000). The risks involved in introducing exotic biological control agents have been debated for many years and some workers have suggested that enhancing populations of indigenous species, rather than introducing exotic ones should have lower risks (Lynch *et al.* 2002).

Public concerns for the environment brought environmental issues associated with the release of new biological control agents to the fore (Harrison *et al.* 2005). Therefore, such releases are now required to follow long procedures and undergo thorough scrutiny by regulatory agencies and also by the public, including the involvement of Maori cultural concepts in New Zealand. As a result, laws have become more and more stringent and the introduction of new biological control agents into the environment is currently a complex and costly process.

In contrast, conservation biological control (CBC) probably poses lower risks compared with Classical BC (Chapter 1). CBC focuses on increasing the efficacy of existing biological control agents by the provision of resource subsidies (Phillips *et al.* 1998; Tylianakis 2001; Kean *et al.* 2003; Gurr *et al.* 2005) and therefore poses fewer risks to the environment. However, the provision of floral resources involving exotic plants leads to the possibility of those plants becoming weeds. Moreover, intra-guild predation is also of increasing interest among invertebrate ecologists (Polis *et al.* 1989). It remains untested whether CBC can disrupt the structure of natural enemy guilds.

This thesis examined whether CBC could enhance the efficacy of an introduced Classical BC agent (*M. hyperodae*) of *L. bonariensis*. Estimated damage to the pasture industry by *L. bonariensis* was between NZ$78 - 251 million per year (Prestidge *et al.* 1991). The damage caused by *L. bonariensis* to pasture declined with the introduction *M. hyperodae* in 1991 (Goldson *et al.* 1992, 1998b). The financial gain derived from the action of this biological control agent is probably more than the suggested NZ$50 million per year (C. B. Phillips, pers. comm.). However, it is clear that *M. hyperodae* has not yet exerted the optimal control of the first summer generation of *L. bonariensis* (Chapter 1). It has been suggested that the provision of resources subsidies for *M. hyperodae* in the field during November and December could increase the first summer generation parasitism of *L. bonariensis* (Phillips *et al.* 1998). Therefore, this thesis research was very timely because enhancing the efficacy of *M. hyperodae* could increase the economic benefit of biological control of this very important pest.

The main aim of this study was to find an appropriate flowering plant that could increase the efficacy of *M. hyperodae* in pastures. To achieve this, a number of experiments were conducted. First, seven flowering plant species were screened in the laboratory to select the best for *M. hyperodae* (Chapter 2). Second, lifetime sugar dynamics were investigated in the laboratory to help understand the effect of feeding on the physiology of *M. hyperodae* (Chapter 3). Third, the effect of buckwheat on the abundance of *M. hyperodae* was investigated to assess parasitoid attraction to flowers in the field (Chapter 4). Fourth, sugar analysis of field collected *M. hyperodae* was used to assess parasitoid feeding history in the field (Chapter 5). Finally, parasitism rates of *L. bonariensis* were measured in relation to the presence and absence of buckwheat in the field (Chapter 6).

Only two plant species, buckwheat (*Fagopyrum esculentum*) and coriander (*Coriandrum sativum*) significantly increased the longevity of *M. hyperodae* in the laboratory (Chapter 2). The head-width of *M. hyperodae* was greater than the corolla aperture of white clover (*Trifolium repens*) and of red clover (*T. pratense*) and, therefore, it was easy to understand the lower performance of the parasitoid with those two plant species. The floral architecture of alyssum (*Lobularia maritima*) and phacelia (*Phacelia tanacetifolia*) did not allow the parasitoids to access nectar sources. White mustard (*Sinapis alba*) had a non-significant positive effect on the longevity of *M. hyperodae*.

Many parasitoids tested with flowering plant species in the laboratory exhibited significantly increased longevity with buckwheat (Tylianakis 2001; Berndt *et al.* 2002; Lavandero *et al.* in press-b). In addition, many field experiments have shown that buckwheat has a positive effect on the efficacy of parasitoids (Heimpel & Jervis 2005). Heimpel *et al.* (2005) reinforced this by suggesting that buckwheat is emerging as a model plant to increase parasitoid efficacy in CBC studies. Some studies have shown that the architecture of the buckwheat flower enables easy access to its nectar sources (Jervis *et al.* 1993; Patt *et al.* 1999; Winkler *et al.* 2003). Results from the present study were consistent with those studies and the larger corolla aperture and shallow corolla depth helped *M. hyperodae* to benefit form buckwheat nectar. Other floral resources have helped to increase the longevity of other parasitoids in the laboratory. For example, phacelia and alyssum gave significant increases in the longevity of some parasitoids (Berndt & Wratten 2005; Lavandero *et al.* in press-a). However, the present study showed that only buckwheat and coriander significantly increased longevity of *M. hyperodae* compared with water.

None of those other studies was able to describe the underlying reasons for the difference between flowering plant species in relation to parasitoid longevity. All those studies used ranking methods, based solely on parasitoid longevity, and the highest-ranked plant was typically used in further experiments. In the current work, nectar analysis work showed that the sucrose/(glucose+fructose) (s/(g+f)) ratio of the nectar sources had a strong effect on *M. hyperodae* longevity. This finding was consistent with Baker and Baker (1983), who showed that parasitoids prefer sucrose-dominant floral nectar. An analysis of phacelia nectar supported this hypothesis because its s/(g+f) ratio was similar to that of buckwheat. *M. hyperodae* did not benefit from phacelia nectar, probably due to difficulty in accessing the flower. However, many other insects benefit from this plant both in the laboratory and field (Holland *et al.* 1994; White *et al.* 1995; Hickman & Wratten 1996; Baggen *et al.* 1999; Gurr *et al.* 2000), although some of these studied insects used mainly its pollen.

Significant differences in sugar levels between fed and unfed *M. hyperodae* were observed in the laboratory (Chapter 3). The common insect haemolymph sugar, trehalose was never detected in unfed parasitoids, but was detected in fed *M. hyperodae* in small quantities. To a certain extent, this is consistent with the findings of Steppuhn & Wäckers (2004) in that they did not observe trehalose in either fed or unfed *Cotesia glomerata*. It appears that fed *M. hyperodae* is able to synthesise small quantities of trehalose from basic sugars. The cause of death appeared to be low sugar levels in unfed parasitoids, but this was not the case in fed

ones (Chapter 3). Glucose was assimilated more quickly than fructose in aged parasitoids. This was not detected in other parasitoids studied for their lifetime sugar levels (Olson *et al.* 2000; Steppuhn & Wäckers 2004; Casas *et al.* 2005). The knowledge of sugar physiology of hymenopteran parasitoids is still at an early stage and requires more research. It may not be possible to extrapolate to hymenopteran parasitoids from other insect groups which have been studied in the past.

The total sugar level and fructose to total sugar (f/t) ratio provided an important tool to discriminate between fed and unfed parasitoids, and had never previously been used for this purpose. The only ratio previously used for any parasitoid in this way was the glucose/(glucose+fructose) (g/(g+f)) ratio (Steppuhn & Wäckers 2004). This ratio was not useful for *M. hyperodae* because of the rapid decrease in glucose levels associated with parasitoid ageing (Chapter 5). Nonetheless, it was possible to assess the benefit of CBC for natural enemies in the field using both the total sugar level and the f/t ratio. Higher f/t ratios in parasitoids up to 6m from buckwheat plots reflected feeding on buckwheat nectar in the field. The distance over which buckwheat influences parasitoid diet is clearly small compared with the scale of pasture fields. The data suggest that buckwheat plots have to be located no more than 12m apart for optimal results.

It was possible to link nectar feeding from buckwheat with parasitoid gut sugar levels in the laboratory (Chapter 3) and also in the field (Chapter 5). The gut sucrose digestion time appeared to be around 1h in both laboratory- and field-tested parasitoids. This is consistent with the gut sugar retention time of *Anaphes iole* Girault (Hymenoptera: Mymaridae) (Willimams *et al.* 2005). The concept of 'signature sugars' has been used for field-collected parasitoids to show they had fed from honeydew (Wäckers *et al.* 2005). Similarly, parasitoids captured near buckwheat plots immediately after feeding have been used to show the link between buckwheat feeding and parasitoid sugars. However, none of previous studies showed a direct link between floral nectar and body sugars of parasitoids. This study was able to show such a link and this should be useful in helping future studies to understand parasitoid feeding sources in the field.

In addition to parasitoids near buckwheat being better fed, there was a significant increase in parasitoid numbers near buckwheat plots. This could be due to both, increased parasitoid longevity from buckwheat feeding, and due to buckwheat attracting parasitoids (Chapter 4) from the surrounding pasture. The latter group are also likely to be benefited from buckwheat

and increased their longevity. Many other field studies also have shown that parasitoids can be abundant near buckwheat (e.g., Irvin *et al.* 2000; Berndt *et al.* 2002). Parasitoids use visual (Wäckers 2004) and olfactory (Takasu & Lewis 1996) cues to locate food sources, such as floral nectar. Therefore it is assumed that a greater number of parasitoids could be expected in the presence of flowers (van Emden 1962; Chaney 1998; Stephens *et al.* 1998). Buckwheat plots at both field sites were attracting parasitoids from 7 to 8m away (Chapter 4).

The size of the buckwheat plots at Field Sites 1 and 2 was $69m^2$ and $20m^2$, respectively. The number of parasitoids attracted to buckwheat plots from 7 to 8m away appeared to differ between these sites; the numbers were very low at at Field Site 1 compared with Field Site 2 (Chapter 4). This suggests that the larger buckwheat plots attracted parasitoids more strongly than smaller plots. However, the size of the plots had no observed effect on parasitoids other than at the 7 – 8m range. Therefore, this study shows the relationship between aggregation and the size of the floral patch. The increased abundance of *M. hyperodae* and better feeding near buckwheat were reflected in higher parasitism of *L. bonariensis* as expected.

The higher parasitism rates observed in the first summer generation of weevils in the buckwheat treatment confirm the hypothesis of Phillips *et al.* (1998) who suggested that the provision of appropriate resources to overwintered *M. hyperodae* during mid November to mid December should increase the parasitism rate of the first summer generation of *L. bonariensis* in mid January. The present study showed that the provision of buckwheat in synchrony with parasitoid adult emergence increased parasitism rates in the field. Phillips *et al.* (1998) further hypothesised that *M. hyperodae* generally achieves its maximum potential in suppressing the second summer generation of *L. bonariensis* and provision of additional resources would not make any difference to parasitism rates. Results from the current work were consistent with this hypothesis because the parasitism rates recorded near buckwheat were not significantly higher in late summer from the second week of February to the last week of April. *M. hyperodae* adults from overwintered generation require additional resources in the field to live longer and encounter more first summer generation *L. bonariensis* adults. However, although the provision of floral resources for overwintered parasitoid adults was effective, it did not make any difference to the second-generation adult weevils' parasitism rates (Chapter 6). Therefore, it seems that sowing buckwheat in late September would be sufficient to achieve the main aim of this work.

7.1 Potential drawbacks of buckwheat and other candidate plant species

Introducing floral resources to agricultural landscapes could benefit other trophic levels in addition to the targeted natural enemies. Ideally, only the target parasitoids should benefit from CBC programmes, or at least they should benefit more than the pest. The floral resource itself should not become a weed, nor should it help fourth trophic-level antagonists for example hyperparasitoids), which may reduce the parasitoid population. The trophic levels of major concern include 1) the crop (grass), 2) the pest, *L. bonariensis* (herbivore), 3) the parasitoid, *M. hyperodae* (natural enemy) and 4) the antagonists (hyperparasitoids) (modified from Gurr *et al.* 2000).

In most of New Zealand, winter frosts prevent regeneration of buckwheat from overwintered seed (Bowie *et al.* 1995) and, therefore, there is no risk of buckwheat becoming a weed in pasture. The provision of floral resources benefited *M. hyperodae* in pasture and increased the efficacy of parasitoids. Consumption of buckwheat pollen in the laboratory by *L. bonariensis* was reported to be very low and it was suggested that buckwheat could be well suited for the present study system (Urrutia 2005). Therefore the risk of benefiting *L. bonariensis* by providing buckwheat is very low. Finally, fourth trophic level parasitoids do not exist in the *M. hyperodae* system in New Zealand pasture, although at least one hyperparasitoid of *M. hyperodae* has been reported in South America (C. B. Phillips pers. comm.). All this suggests that *M. hyperodae* is likely to benefit more from CBC using buckwheat than organisms at any other trophic level.

7.2 Likelihood of uptake of these protocols in pasture and other systems

This research has demonstrated the potential of CBC in New Zealand pasture, albeit at a relatively small spatial scale. There are many steps required before of the outcome of this study can be applied at a commercial scale. First, the appropriateness of buckwheat in pastures is questionable. The height of the plant appears to be not compatible with the height of pasture plants such as grass and clover species. Second, protecting buckwheat plants from livestock would be costly for farmers. Third, for the provision of buckwheat to be effective in pasture, patches would ideally be 12m apart. This deployment of buckwheat in pasture could alter the traditional farming landscape and, therefore, may not be acceptable to farmers. However, growing buckwheat in smaller paddocks along existing fence lines would reduce the difficulty of allocating pasture land to buckwheat and minimise the costs involved in

protecting buckwheat from livestock. It could also be possible to grow buckwheat along fence lines around large paddocks as well, and this may increase parasitism rates to a limited spatial extent.

7. 3 Conclusions

There are numerous pests in pasture (Chapter 1) that are responsible for causing heavy losses to pasture production every year. Suppressing those pests through CBC could help farmers to increase their productivity. The timely provision of floral resources in the field in this work increased parasitoid efficacy and, thus, increased the parasitism rates of the pest.

This study has shown that the there are a number of flower qualities required for *M. hyperodae* to feed from nectar sources. The 'right' floral architecture and nectar quality are the most important features. Therefore, more research should be conducted to identify appropriate floral resources that have these qualities. In addition flowering plants should have agronomic compatibility with pasture plants. There may be potential in evaluating the potential of native New Zealand plants to fulfil these requirements.

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